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Determination of Corticosteroids in Fish Plasma by High Performance Liquid Chromatography: Evaluation of the Method Using Striped Bass (*Morone saxatilis*)

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MacFarlane, R. B. 1984. Determination of corticosteroids in fish plasma by high performance liquid chromatography: evaluation of the method using striped bass (*Morone saxatilis*). Can. J. Fish. Aquat. Sci. 41: 1280–1286.

A sensitive, specific, and reproducible technique for the simultaneous determination of cortisol, cortisone, and corticosterone in fish plasma was developed using high performance liquid chromatography (HPLC). Corticosteroids were extracted from plasma by methylene chloride and Sep-Pak cartridges for comparative purposes. The Sep-Pak method proved more convenient and efficient. Chromatography was accomplished on a nitrile bonded phase using a methanol–water mobile phase. Steroids were identified and quantified by monitoring absorbance at 254 and 280 nm simultaneously. Sensitivity is approximately 5 ng for each steroid. An evaluation of the technique using subadult striped bass (*Morone saxatilis*) revealed that cortisol was the only corticosteroid detected. Plasma cortisol concentrations increased in response to brief confinement and crowding, supporting the efficacy of this measurement as an evaluator of stress in striped bass. The HPLC technique provides specific determination of each corticosteroid (thus eliminating cross-reactivity problems inherent in radioimmunoassay methods), resolves corticosteroids from androgens and estrogens, and permits the isolation and purification of individual steroids.

À l'aide de la chromatographie en phase liquide moderne (CPLM), on a mis au point une technique sensible, précise et reproductible pour la détermination simultanée du cortisol, de la cortisone et de la corticostérone dans le plasma du poisson. Des corticostéroïdes ont été extraits du plasma, à des fins de comparaison, au moyen de dichlorométhane et de cartouches Sep-Pak. La méthode Sep-Pak s'est révélée la plus convenable et la plus efficace. La chromatographie a été effectuée avec une phase fixe sur nitrile et une phase mobile constituée d'eau et de méthanol. On a identifié et quantifié les stéroïdes en notant l'absorption à 254 nm et à 280 nm simultanément. La sensibilité s'élève à environ 5 ng pour chaque stéroïde. Une évaluation de la technique effectuée sur des bars rayés (*Morone saxatilis*) presque adultes a révélé que le cortisol était le seul corticostéroïde décelé. Les concentrations de cortisol dans le plasma augmentaient en réaction à la courte stabulation et à l'entassement, ce qui étaye l'efficacité de cette quantification comme mesure du stress chez le bar rayé. La technique de CPLM fournit une détermination précise de chaque corticostéroïde (ce qui élimine les problèmes de réactivité croisée, particuliers aux épreuves de radio-immunité), sépare les corticostéroïdes des androgènes et des estrogènes et permet l'isolation et la purification de chaque stéroïde.

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There is considerable evidence that corticosteroids participate in, or regulate, many physiological and biochemical processes in fish. According to the General Adaptation Syndrome (Selye 1950), corticosteroid production is a primary response in vertebrates to stressors and promotes various secondary metabolic responses in an attempt to adapt the organism to altered environmental conditions. Elevated plasma corticosteroid concentrations have been reported in fish exposed to physical stressors (Mazeaud et al. 1977; Strange et al. 1977; Barton et al. 1980), social interactions (Ejike and Schreck 1980; Peters et al. 1980), and pollutants (Hill and Fromm 1968; Donaldson and Dye 1975; Thomas et al. 1980). The evaluation of physiological stress could provide a basis for understanding reductions in population growth and reproduction in systems subjected to environmental alteration. An assessment of stress is, therefore, of particular importance in fish aquaculture where extensive environmental manipulations are employed to provide optimum yield, often leading to stress-induced problems (Pickering 1981).

If the objective of a study is to measure the total corticosteroid response of fish to particular conditions, current widely practiced techniques such as radioimmunoassay (RIA), competitive protein binding (CPB), and fluorometry are acceptable. However, if the objective is to determine specific corticosteroids produced by a particular species, or when investigating steroid biosynthetic and degradative pathways, these techniques have limitations. For example, they may lack specificity, reproducibility, or convenience. Whereas RIA is more specific for a

particular steroid, for example cortisol, than is the CPB assay (Farmer and Pierce 1974), commercially available antisera display considerable cross-reactivity with other steroids (Leach and Taylor 1977; Thomas et al. 1980). To validate these methods for assaying a particular steroid, or when measuring corticosteroid metabolic sequences, laborious techniques such as thin-layer chromatography must be employed; these often lack quantitative accuracy and precision.

Recently, high performance liquid chromatography (HPLC) techniques have been developed and used extensively in biomedical applications for the determination of corticosteroids in mammalian fluids (Kawasaki et al. 1979; Rose and Jusko 1979) and tissues (O'Hare et al. 1976). These techniques overcome many of the limitations inherent in the other analytical methods. Despite widespread use with mammals, there have been no reports documenting HPLC determinations of corticosteroids in fish.

My objective was to develop and evaluate an HPLC method for the simultaneous determination of cortisol, cortisone, and corticosterone in fish plasma; these are considered to be the predominant glucocorticoids in teleosts (Idler and Truscott 1972; Butler 1973). I used striped bass (*Morone saxatilis*) plasma for method evaluation. Corticosteroids were measured in resting controls and in fish subjected to confinement and crowding, simulating conditions that may occur during intensive culture or experimentation.

Materials and Methods

Chromatography

The HPLC system consisted of a model 6000A solvent delivery system, an M-45 solvent delivery system, a U6K injector, a model 720 system controller, a model 730 data module, and a model 440 UV absorbance detector with dual wavelength capability (Waters Assoc., Inc., Milford, MA).[1] The detector was equipped to monitor 254 and 280 nm wavelengths simultaneously. The corticosteroids were separated on a Radial-Pak CN cartridge, 8 mm ID × 10 cm long, in a Radial Compression Module (RCM-100, Waters Assoc., Inc.) using an isocratic mobile phase of 30% methanol: 70% water delivered at a flow rate of 3 mL/min. Solvents for the mobile phase were purchased from Burdick and Jackson Laboratories, Muskegon, MI, and were filtered and degassed by vacuum prior to use.

Corticosteroids were identified by a comparison with retention times or capacity factors (k'), defined by $k' = (t_r - t_0)/t_0$ where t_r is the retention time (minutes) of the steroid and t_0 is the retention time (minutes) of unretained components (i.e. solvent front), of steroid standards. For additional confirmation, the absorbance ratio of 254 to 280 nm of the steroids extracted from plasma was compared with that of steroid standards. Quantification of corticosteroids was based on peak heights that were directly proportional to the quantity of steroid injected ranging from 5 ng to at least 1 μg.

All steroid standards were purchased from Sigma, St. Louis, MO). Primary cortisol (F), cortisone (E), and corticosterone (B) standards were prepared in methanol at 40 μg/mL for each steroid and stored at −60°C. Working standards were prepared by diluting the primary standards with methanol to produce concentrations ranging from 5 to 400 ng of each compound per 80 μL. Standard solutions of androgens, estrogens, progesterone, and 4-pregnene-17α,20β-diol-3-one were prepared similarly and evaluated for potential interference with corticosteroid determinations.

Corticosteroid Extraction

Corticosteroids were extracted from plasma by two methods. Initially, methylene chloride was used. Plasma samples were warmed to 4°C (if frozen) at which time 1.0 mL was withdrawn from each sample and transferred to a 12-mL centrifuge tube. An appropriate quantity of internal standard (IS) was added, if this method was used. Then, 0.1 mL of 2 mol NaOH/L and 7.0 mL of cold methylene chloride (4°C) were added, followed by vigorous stirring for 1 min in a vortex-type mixer. The tubes were centrifuged for 10 min to aid in phase separation, and the aqueous layer was removed with a Pasteur pipette. Exactly 6.0 mL of the organic phase was transferred to a clean tube and evaporated to dryness under a stream of nitrogen. For maximum recovery it is essential that during the evaporation the walls of the tubes are washed frequently with methylene chloride. Finally, the residue was dissolved in 100 μL of methanol, 80 μL of which was injected into the chromatograph. Both the methylene chloride and methanol were HPLC grade.

More recently, to conserve solvent, to eliminate the use of hazardous methylene chloride, and to compare recovery efficiency, Sep-Pak C_{18} cartridges (Waters Assoc., Inc., Milford, MA) were evaluated for steroid extraction from plasma. Cartridges were conditioned with 2.0 mL of methanol, followed by 5.0 mL of water as suggested by the manufacturer. Plasma samples were diluted 1:1 with water to reduce viscosity and passed through the cartridges at about 1 drop/s. Cartridges were washed with 3.0 mL of 20% methanol. The corticosteroids were eluted with 2.0 mL of methanol, which was subsequently evaporated under nitrogen, with frequent methanol washes of the tube. Residues were reconstituted and injected the same as with the methylene chloride extraction technique.

To determine the efficiencies of the extraction procedures, plasma from each of four to six fish was separated into three portions. For the solvent extraction method, portions received either 100 or 500 ng of each steroid. The third fraction received no added steroids; hence, quantities found represented plasma levels of corticosteroids in unstressed striped bass.

To evaluate the Sep-Pak technique, ^{14}C-radiolabeled cortisol (specific activity 2.0 GBq/mmol, New England Nuclear, Boston, MA) and corticosterone (specific activity 1.9 GBq/mmol, Amersham Corp., Arlington Heights, IL) were prepared in methanol and added to plasma. Since cortisone is intermediate in polarity between cortisol and corticosterone, and radiolabeled cortisone is not generally available, it was not evaluated. Cortisol recovery efficiency was assessed by adding 600 and 60 ng to portions of plasma. Similarly, 200 and 20 ng of corticosterone were added to separate plasma aliquots. Portions of plasma without radiolabeled steroids were also processed to correct for the potential of interfering compounds that might quench scintillation counts. All eluting fractions (i.e. diluted plasma, wash, and methanol) were collected separately in scintillation vials and evaporated with nitrogen; 15 mL of Aquasol (New England Nuclear) was added to each vial. Vials were counted in a Packard Tri-Carb model 2211 liquid scintillation counter (Packard, Downers Grove, IL). Counting efficiencies were determined by the sample channels ratio method for quench correction, and steroid recovery efficiencies

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service.

were calculated from dpm's obtained from samples and dpm's obtained from vials containing spike concentrations of [^{14}C]cortisol or [^{14}C]corticosterone.

In addition to assessing recovery efficiencies of Sep-Pak extractions immediately following plasma collection, several plasma samples were spiked with radiolabeled cortisol or corticosterone and passed through cartridges, which were then capped with Teflon tape and placed in the refrigerator for 1 mo prior to elution and counting.

Since fish plasma may contain other steroids, such as androgens and estrogens, in measurable concentrations during certain states of sexual maturity that may interfere with corticosteroid determination, capacity factors of other steroids reported in fish were assessed. Plasma was stripped of all steroids by elution through a conditioned Sep-Pak C_{18} cartridge. Approximately 200 ng each of testosterone, 11-ketotestosterone, estriol, estrone, 17β-estradiol, progesterone, and 4-pregnene-17α,20β-diol-3-one were added separately to triplicate 1.0 mL aliquots of plasma. Steroids were extracted using the Sep-Pak technique, evaporated under nitrogen, reconstituted with methanol, and chromatographed by the same procedure used for corticosteroids.

Method Evaluation

Plasma for method development and evaluation was collected from subadult striped bass, *Morone saxatilis* (2-yr-old, mean SL 21 ± 4 cm). Fish were collected from the San Francisco Bay–Delta estuary by gill net during July and August 1981 and acclimatized for at least 1 mo to laboratory conditions at the Steinhart Aquarium, San Francisco, CA. They were maintained in a semi-open, recirculating seawater system in 800-L fiberglass aquaria receiving filtered seawater (temperature 17.8°C, salinity 32.9‰) under ambient light conditions.

Blood was collected by cardiac puncture in sterile Vacutainers containing either EDTA or heparin. All fish were bled within 3 min (usually within 2 min) of initial perturbation to control for capture-induced corticosteroid production. The blood was immediately centrifuged at 1600 × *g* and the plasma was either extracted immediately or stored at −60°C until extraction.

To assess the corticosteroid response of striped bass subjected to confinement and crowding, I placed six fish in a large dip net in an aquarium for a 30-min duration prior to blood sampling and extraction with methylene chloride. Another group of seven fish was treated similarly, but the plasma was extracted by the Sep-Pak technique using corticosterone as an internal standard. Unconfined controls were sampled concurrently using both extraction techniques.

Results and Discussion

Chromatography

The HPLC technique provided a good separation of cortisol, cortisone, and corticosterone with an analysis time of less than 15 min (Fig. 1). Using this chromatographic technique, plasma steroid quantities as low as 5 ng can be separated and accurately quantified. Thus, the minimum volume of plasma required is controlled by that quantity that will result in at least 5 ng of steroid in the solution injected into the chromatograph. This sensitivity is comparable with that obtained by RIA (Leach and Taylor 1977) and CPB (Strange et al. 1977) techniques. However, the HPLC technique provides specific determination

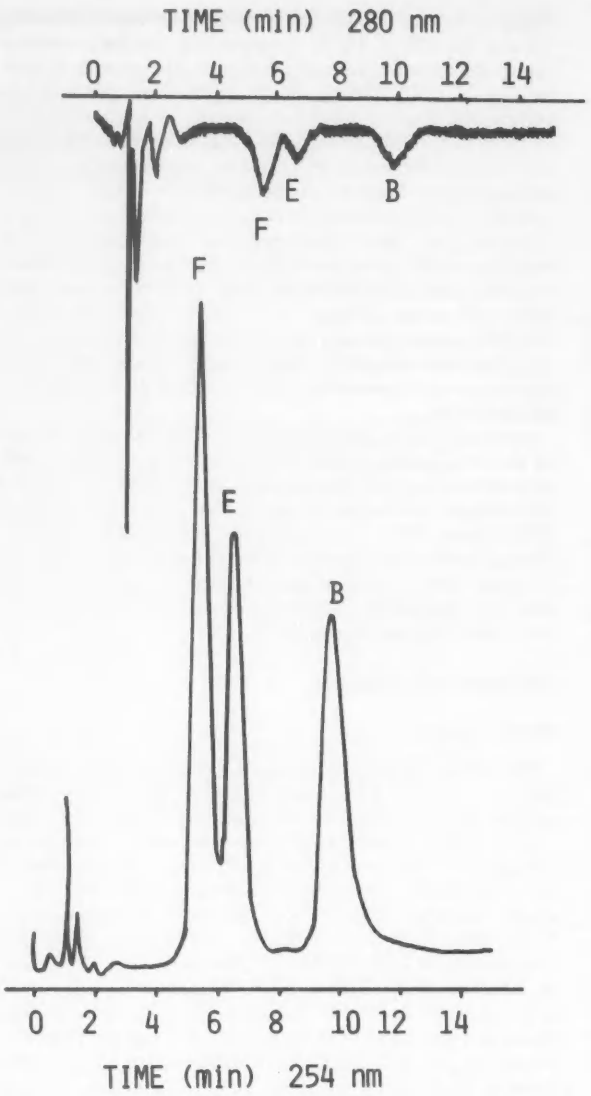

FIG. 1. Typical chromatogram of the separation of 200 ng of cortisol (F), cortisone (E), and corticosterone (B) standards. Bottom profile is absorbance at 254 nm, 0.010 absorbance units full scale (aufs). Top profile (inverted) is absorbance at 280 nm, 0.005 aufs.

of each steroid. The other methods are incapable of this specificity unless prior study confirms that the steroid being assayed is the only one present in all samples.

Capacity factors (k') were within the optimum range of 1–10 (Snyder and Kirkland 1979) for all corticosteroids and were highly reproducible (Table 1). The speed and resolution of this chromatographic system is partly attributable to the RCM-100 technology. These compounds could be adequately resolved using conventional 25-cm stainless steel columns packed with a suitable CN-bonded phase. However, the retention times would be much longer, resulting in fewer analyses per day. Additionally, stainless steel columns are more expensive than Radial-Pak cartridges.

TABLE 1. Corticosteroid capacity factors (k')[a] and absorbance ratios (254/280 nm) using a 30% methanol in water mobile phase at a flow rate of 3 mL/min through a CN-bonded phase Radial-Pak cartridge (SE in parentheses).

Corticosteroid	n	Capacity factor (k')	Absorbance ratio (254/280 nm)
Cortisol (F)	18	4.26 (0.01)	21.3 (0.3)
Cortisone (E)	17	5.25 (0.02)	31.5 (0.9)
Corticosterone (B)	17	8.22 (0.04)	20.6 (0.8)

[a]Elution time of unretained solutes, $t_0 = 1.04$ min.

The detector response at 254 nm was linear over a range of 0 to at least 1 μg for each steroid. Linearity was periodically assessed by injecting quantities ranging from 5 to 200 ng (Fig. 2). Responses were highly reproducible, with cortisol producing a greater response than either cortisone or corticosterone, due to spectral characteristics and earlier elution time.

During the method development, a number of other chromatographic conditions were evaluated. Separations were attempted using both octyl silane (C-8) and octadecyl silane (C-18) bonded phases with either methanol–water or acetonitrile–water mobile phases. In all cases these conditions resulted in reduced resolution of cortisol and cortisone. Also, the retention time for corticosterone was much longer using an isocratic mobile phase. To reduce the analysis time, a gradient of increasing organic component concentration had to be used that caused an elevation of the baseline resulting in difficult quantification of low levels of corticosterone.

Simultaneous monitoring of the absorbance at 254 and 280 nm is useful as an added measure of specificity for corticosteroid identification (Table 1). Cortisol, cortisone, and corticosterone have maximum absorbance at about 240 nm in methanol but show considerable absorbance at 254 nm. This is a common wavelength of all HPLC absorbance detectors and therefore increases the general applicability of the assay technique. Corticosteroids have little absorbance at 280 nm in methanol (Fig. 1). Absorbance ratios aid in peak identification, particularly in the early part of the separation. In many cases, methylene chloride extracts of plasma contain compounds that elute in the first 6 min of the separation that have low 254/280 nm absorbance ratios due to high absorbance at 280 nm. Without 280 nm absorbance values these peaks may be incorrectly identified as corticosteroids. These interfering peaks may be lipoproteins, since lipids are present in greater concentrations in the plasma on many fish species relative to higher vertebrates (Lewander et al. 1974; Larsson et al. 1976).

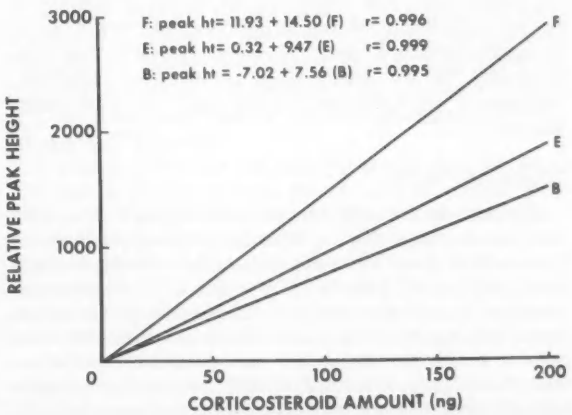

FIG. 2. Representative standard curves for injected quantities of corticosteroids ranging from 5 to 200 ng.

Corticosteroid Extraction

Experiments assessing the efficiency of the solvent extraction procedure revealed that cold methylene chloride greatly reduced the quantities of interfering compounds coextracted with the corticosteroids compared with methylene chloride used at room temperature. This modification, suggested by D. R. Idler (Memorial University of Newfoundland, St. John's, Nfld. A1C 5S7, pers. comm.), improved the resolution of cortisol particularly when low levels were present in plasma.

The recovery of steroids from striped bass plasma by solvent extraction ranged from about 42% for cortisone to 64% for corticosterone and was independent of concentration (Table 2). The recovery efficiency for cortisol (62–63%) was similar to that reported for RIA assays in some laboratories (Sangalang et al. 1980; Thomas et al. 1980) but lower than in others (Weisbart and Jenkins 1981). Although the recoveries were relatively low when compared with studies conducted on mammalian plasma (Rose and Jusko 1979; De Vries et al. 1980), the reproducibility, reflected by the standard error values, was good.

The use of Sep-Pak C_{18} cartridges greatly simplified the extraction and analysis of plasma steroids. In most cases, the large quantities of interfering compounds, evident in methylene chloride extracts eluting during the initial portion of the chromatographic separations, were diminished by the cartridge extraction technique (Fig. 3). This improved the detection limit and quantification of cortisol significantly. Additional sensitivity was provided by the increased recovery efficiency of the Sep-Pak cartridges (Table 3). Summation of radioactivity in all eluted fractions indicated that recovery was quantitative with very little loss in the 20% methanol wash fraction or retention on the packing. The first 2.0 mL of methanol eluted greater than 96% of the retained steroids; however, t-tests revealed that for each compound there was a significant decrease in recovery of steroids at the lower concentration ($P < 0.001$).

Another advantage of the Sep-Pak technique is that plasma

TABLE 2. Recovery of corticosteroids added to striped bass plasma using methylene chloride extraction method. Values are means of five fish (SE in parentheses).

Corticosteroid	Added (ng)	Recovery (ng)	Recovery (%)
Cortisol	0	32.5 (0.5)	—
	100	83.4 (1.4)	63.0
	500	331.3 (6.8)	62.2
Cortisone	0	0	—
	100	46.3 (0.7)	46.3
	500	209.6 (5.9)	41.9
Corticosterone	0	0	—
	100	61.7 (1.2)	61.7
	500	321.1 (5.0)	64.2

TABLE 3. Recovery of ^{14}C-labeled corticosteroids added to striped bass plasma using Sep-Pak C_{18} cartridges (SE in parentheses).

Corticosteroid	Added (ng)	n	% total recovery	% recovery in each fraction: Sample eluant	3 mL of 20% MeOH	First 2 mL of MeOH	Second 2 mL of MeOH
Cortisol	600	6	97.0 (1.3)	0.4 (0.11)	0.2 (0.03)	98.9 (0.08)	0.4 (0.06)
	60	4	98.9 (0.4)	0.7 (0.20)	0.7 (0.02)	97.5 (0.16)	1.1 (0.34)
Corticosterone	200	4	97.8 (0.2)	0.4 (0.24)	0.1 (0.02)	98.6 (0.11)	0.9 (0.31)
	20	4	99.7 (0.3)	1.3 (0.50)	0.9 (0.14)	96.3 (0.32)	1.6 (0.30)

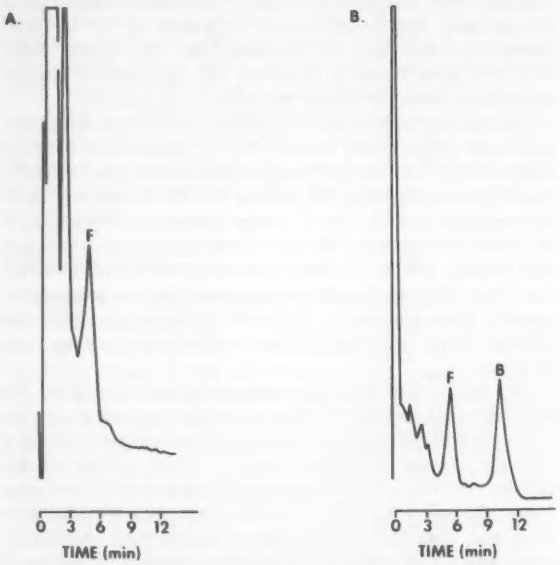

FIG. 3. Chromatograms at 254 nm of plasma extracts of cortisol (compound F) by (A) methylene chloride and (B) Sep-Pak C_{18} cartridge. Corticosterone (compound B) is internal standard added to plasma prior to extraction. Vertical scale, 0.005 aufs.

TABLE 4. Capacity factors (k') of androgens, estrogens, progesterone, and 4-pregnene-17α,20β-diol-3-one using chromatographic conditions specified in Table 1. Data based on retention times of steroids added to stripped plasma. Each value is the mean of three determinations (SE in parentheses).

Steroid	Capacity factor (k')
Androgens	
11-Ketotestosterone	6.54 (0.03)
Testosterone	17.28 (0.05)
Estrogens	
Estriol	5.69 (0.02)
β-Estradiol	19.34 (0.05)
Estrone	20.81 (0.06)
4-Pregnene-17α,20β-diol-3-one	15.75 (0.05)
Progesterone[a]	34.71 (0.15)

[a] k' for progesterone produced by linear increase in methanol concentration in mobile phase from 30 to 40% between 25 and 35 min postinjection.

samples can be extracted and the cartridges stored for at least 1 mo in the refrigerator. Recovery of radiolabeled cortisol and corticosterone was greater than 94 and 91%, respectively. These cartridges proved to be highly convenient and efficient for steroid extraction from plasma, as well as versatile. Shackleton and Whitney (1980) obtained similar results for urinary steroid extractions, and Whitney and Thaler (1980) demonstrated that the cartridges were effective for recovery of bile salts in serum, amniotic fluid, and urine.

During advanced states of sexual maturity, plasma may contain steroids other than cortisol, cortisone, and corticosterone that may interfere (i.e. have similar capacity factors) with the chromatographic determination of the corticosteroids. Other steroids reported in fish include the androgens, testosterone and 11-ketotestosterone; the estrogens, 17β-estradiol, estriol, and estrone; progesterone; and 4-pregnene-17α,20β-diol-3-one (Schmidt and Idler 1962; Chester Jones et al. 1974; Fontaine 1975). The Sep-Pak technique efficiently extracted these compounds from spiked plasma samples previously stripped of all steroids. Chromatograms of these extracts revealed that, except for estriol, all were well resolved from cortisol, cortisone, and corticosterone (Table 4). Estriol has a capacity factor similar to cortisone but does not interfere with the quantification of cortisone because it has a 254/280 nm absorbance ratio of approximately 0.13 due to very low absorbance at 254 nm. Estriol, estrone, and 17β-estradiol differ from the other steroids evaluated in that they have maximum absorbance at 280 nm and very little absorbance at 254 nm. Estrogen quantities less than 200 ng are undetectable at 254 nm, 0.005 absorbance units full scale, and are therefore unlikely to interfere in plasma corticosteroid determinations. In fact, due to the good resolution of these compounds, this HPLC technique is suitable for the determination of androgens, estrogens, progesterone, and 4-pregnene-17α,20β-diol-3-one in fish plasma and tissues.

Striped Bass Studies

Cortisol was the only corticosteroid identified in 2-yr-old striped bass plasma (Fig. 3; Table 2). Cortisol is the predominant, and in many cases the only, corticosteroid found in teleosts (Idler and Truscott 1972; Butler 1973). Cortisol was identified by retention time and the 254/280 nm absorbance ratio. The absence of corticosterone, or any other compound with a similar retention time and absorbance at 254 nm, permitted its use as an internal standard to correct for incomplete steroid recovery in subsequent analyses.

Earlier experiments for HPLC method development indicated that cortisol, cortisone, and corticosterone may be present in

plasma of 5-yr-old sexually mature, female striped bass. This was based on a separation using a C-18 column with a methanol–water gradient mobile phase without simultaneous monitoring at 280 nm. Peaks were evident at the retention times of these compounds; however, this alone does not provide sufficient identification.

Although there is little documentation on quantitative and qualitative changes of plasma corticosteroids during different developmental stages in teleosts, there are indications that they occur. Most of this research concerns salmonids during their upstream spawning migrations. What is known has been reviewed by Idler and Truscott (1972). It has been generally reported that plasma corticosteroid levels increase with increasing sexual maturation reaching maximal levels during or subsequent to spawning. Schmidt and Idler (1962) found increases in plasma cortisol and cortisone in *Oncorhynchus nerka* corresponding to sexual development but no significant difference in plasma corticosteroid concentrations in *Salmo salar* between the beginning of the upstream migration and at spawning. Cortisol and cortisone were always the predominant compounds, but in a few individuals, corticosterone was a major component during the early phase of the migration. Cortisone also has been identified in other fish species, particularly those with high total plasma corticosteroid levels (Idler and Truscott 1972). Elevated plasma corticosteroid levels during spawning have been reported in other teleosts including *Coregonus lavaretus* (Fuller et al. 1974) and *Oncorhynchus tschawytscha* (Hane and Robertson 1959); however, no clear trend was evident in *Fundulus heteroclitus* (Leach and Taylor 1977), and mature female goldfish (*Carassius auratus*) had lower cortisol levels than maturing and immature fish (Peter et al. 1978).

Qualitative and quantitative changes in plasma corticosteroids may be due to factors other than sexual maturation such as vigorous exercise during upstream migration and decreased feeding or capture stress. But Sundararaj and Goswami (1966) demonstrated that corticosteroids were capable of inducing ovulation in the catfish, *Heteropneustes fossilis*. Thus the involvement of corticosteroids in sexual maturation in teleosts is unresolved and may be species specific.

Another source of variability in plasma corticosteroid concentration may be sex. Generally, females have been shown to have higher levels during sexual maturation than males in *Oncorhynchus* sp. (Hane and Robertson 1959; Schmidt and Idler 1962; Idler and Truscott 1972) and *Coregonus lavaretus* (Fuller et al. 1974). In my study, there was no significant difference in plasma cortisol levels between male and female subadult striped bass. This profile was also shown for immature *Carassius auratus* (Peter et al. 1978) and *Fundulus heteroclitus* (Leach and Taylor 1977).

Confinement in a net suspended in an aquarium for 30 min resulted in greatly elevated plasma cortisol concentrations (Table 5). Again, cortisol was the only corticosteroid found. Confinement produced a sixfold increase in plasma concentrations of cortisol, indicating the potential use of this factor for assessing stress in this species. Similar changes of plasma cortisol have been recorded for hybrid striped bass (Tomasso et al. 1980), chinook salmon (*Oncorhynchus tschawytscha*) and cutthroat trout (*Salmo clarki*) (Strange et al. 1977), rainbow trout (*Salmo gairdneri*) (Barton et al. 1980), and channel catfish (*Ictalurus punctatus*) (Davies and Parker 1979) under confined conditions.

The HPLC technique presented here provides a versatile procedure for identification and quantification of corticosteroids in plasma or serum of fishes and other aquatic vertebrates. A positive feature of this technique is that many steroids can be assessed simultaneously. The technique is suitable for the determination of corticosteroids, estrogens, and androgens in tissues and biological fluids. In addition to assessing steroidal responses produced by stress, this technique is useful for studying steroid metabolism, production, and clearance rates.

TABLE 5. Plasma cortisol concentrations in striped bass subjected to control conditions or confinement and crowding stressors. Data presented from both plasma extraction methods (SE in parentheses).

Extraction method	*n*	Cortisol (ng/mL) Control	Cortisol (ng/mL) Confined and crowded
Methylene chloride[a]	6	51 (1)	321 (36)
Sep-Pak C_{18}[b]	7	46 (3)	282 (21)

[a]Cortisol concentration corrected for 63% recovery.
[b]Cortisol concentration corrected by recovery of internal standard (corticosterone).

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Variable Gut Residence Time: Problems in Inferring Feeding Rate from Stomach Fullness of a Mysid Crustacean

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The gut residence time of the mysid crustacean *Neomysis mercedis* is extremely variable and is negatively correlated with ingestion rate. In two experiments in which mysids were fed copepod meals followed by continuous exposure to *Daphnia*, passage times varied from less than 1 to more than 13 h, and there were significant negative correlations of both copepod and daphnid passage times with the average feeding rate on *Daphnia*. In a third experiment starved mysids retained significant amounts of material in the stomach for more than 3 d. This dependence of evacuation rate on feeding activity may invalidate attempts to infer absolute or relative ingestion rate from the stomach fullness of field-caught animals.

Le temps de séjour des aliments dans l'intestin du crustacé mysidacé *Neomysis mercedis*, en corrélation négative avec le taux d'ingestion, est extrêmement variable. Dans deux expériences où des mysidacés ont été nourris de copépodes puis exposés continuellement à *Daphnia*, le temps d'élimination variait de moins de 1 h à plus de 13 h. En outre, il y avait des corrélations négatives nettes entre les temps d'élimination des copépodes et des daphnies et le taux moyen d'ingestion de *Daphnia*. Au cours d'une troisième expérience, des mysidacés privés de nourriture ont retenu d'importantes quantités d'aliments dans leur estomac pendant plus de 3 jours. Cette dépendance du taux d'évacuation sur l'activité alimentaire peut invalider les tentatives d'inférence du taux absolu ou relatif d'ingestion en fonction de la plénitude de l'estomac des animaux capturés sur le terrain.

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Ecologists have long sought accurate estimates of the natural feeding rates of predators. Rates can be measured precisely in small-scale laboratory experiments, but the effects of confinement on both predator and prey mean that extrapolation of experimental results to field situations may be misleading (Reeve 1980). Gut contents of field-caught specimens have been used to estimate the in situ feeding rates of many animals, including opisthobranch gastropods (Paine 1965), pseudoscorpions (Phillipson 1960), copepods (Mackas and Bohrer 1976), freshwater mysids (Rybock 1978), euphausiids (Sameoto 1982), stonefly larvae (Allan 1982), caddis larvae (Hildrew and Townsend 1982), chaetognaths (Feigenbaum 1982), and fishes (Persson 1982). Though stomach fullness cannot be unambiguously interpreted without a knowledge of gut residence time, the rate of movement of material through the gut has often been ignored in attempts to infer relative feeding intensity from gut contents. For example, a large literature on the feeding rhythms of euphausiids (early work reviewed by Mauchline and Fisher 1969; Roger 1973, 1975; Hu 1978; Sameoto 1980) has developed in the virtual absence of empirical data on the magnitude and variability of gut residence time (but see Heyraud 1979).

There are theoretical reasons for supposing that animals should be capable of adjusting gut residence times (Lehman 1976; Taghon 1981), and there is evidence for a variety of invertebrates that retention times may be variable. For example, temperature influences gut clearance rates in the amphipod *Gammarus* (Marchant and Hynes 1981) and the mayfly *Hexagenia* (Zimmerman and Wissing 1978); digestion time varies with prey type and temperature in larval *Chaoborus* (Fedorenko 1975); and retention times depend on ambient food concentrations for the cladoceran *Daphnia* (Geller 1975) and certain marine copepods (Arashkevich 1977). My objective was to demonstrate for the mysid crustacean *Neomysis mercedis* that gut residence time varies with ingestion rate and that stomach fullness is therefore an unreliable indicator of in situ feeding rate.

Methods

Experiments 1 and 2 were designed to allow the direct determination of gut passage time in actively feeding mysids. Specimens of *N. mercedis* were collected in the morning from a 20-m-deep station in Lake Washington, Seattle, with an epibenthic dredge (Murtaugh 1981) and transported live to the laboratory in 3.5-L jars of surface water. Healthy mysids were then pipetted into 3-L trays of filtered (50-μm mesh) lake water and held in a darkened incubator at 10°C until the beginning of an experiment.

These two experiments were done on different dates under slightly different conditions (described in Table 1), but the basic design was the same for both. The mysids, which still had dark

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TABLE 1. Conditions of Experiments 1 and 2. Prey lengths, measured in subsamples of the stocks used in the experiments, are from head to end of caudal rami (*Diaptomus*) or head to base of tail spine (*Daphnia*).

	Experiment 1	Experiment 2
Mean length of mysids (and range)	14.1 mm (12.3–15.0 mm, $n = 10$)	12.1 mm (11.6–12.8 mm, $n = 21$)
Time starved before experiment	22 h	21.5 h
First food (density, mean length)	*Diaptomus nevadensis* (one per jar, 3.8 mm)	*Diaptomus sicilis* (five per jar, 1.55 mm)
Length of exposure to first food	15–40 min	18–55 min
Second food (density, mean length)	*Daphnia pulicaria* (2 or 5 per jar, 1.55 mm)	*Daphnia pulicaria* (1, 5, or 10 per jar, 1.18 mm)
Total duration of experiment	10 h	13.5 h

brown material in their stomachs at the beginning of the trials, were transferred individually to 230-mL glass jars into which one or five bright red, diaptomid copepods had been placed (Table 1); red copepods were chosen to provide a conspicuous marker for the observation of gut clearance. The copepods, collected from the saline waters of Soap Lake, Washington, were immobilized in the fresh Lake Washington water and were quickly attacked by the mysids. The jars were kept in the dark at 10°C but were removed at frequent intervals for visual checks. When a mysid had ingested a substantial amount of copepod material (indicated by bright red coloration of the stomach), uneaten copepods and debris were removed and 1, 2, 5, or 10 *Daphnia pulicaria* from Lake Washington cultures were added to the jar (see Table 1). Various prey densities were used in an attempt to get a broad range of ingestion rates.

The jars, each containing one mysid and 1–10 *Daphnia*, were kept in the darkened 10°C incubator. About once an hour the jars were removed and quickly examined under room light; fecal pellets were pipetted off the jar bottom, and any missing *Daphnia* were replaced. Typically, the first pellets were dark brown, representing material in the guts at the onset of the experiment. The passage of the copepod material was marked by the first appearance of bright red fecal pellets. These eventually gave way to light brown pellets representing *Daphnia* remains. I dissected and microscopically examined most pellets to verify this matching of color and prey type. Whereas the separation of the different foods in the fecal strings was not always clear-cut, indicating some mixing of material in the stomach, copepod legs and mouthparts were usually evident in the red pellets, and *Daphnia* mandibles and postabdominal claws were the main identifiable constituents of the light brown material that was last to be egested. The trials were terminated after 10 or 13.5 h (Table 1), when most of the mysids had produced red feces. The mysids were preserved and measured (total body length, from the apex of the rostrum to the apices of the telson), and fecal pellets and prey remaining in the jars were collected.

Gut residence times were the intervals between the first consumption of an item (either copepods or *Daphnia*) and the first appearance of that item in fecal material. Ingestion rates (*Daphnia* per hour) were expressed as the total number of daphnids consumed divided by the time elapsed between the first presentation of *Daphnia* and the termination of the experiment. Egestion rates (cubic millimetres of feces per hour) were calculated from the volume of fecal pellets produced: the length (a) and diameter (b) of all pellets were measured under a dissecting microscope and the volume of material in the cylindrical pellets computed as $\pi ab^2/4$. Stomachs of the experimental animals were dissected out and their percent fullness estimated visually. The stiff-walled, roughly spherical mysid stomach retains its shape even when empty; stomach capacity can be approximated as the volume of a prolate spheroid, $(4/3)\pi a^2 b$, where a is one half of the average of stomach width and height and b is one half of the stomach length. Stomach capacities of the experimental animals were calculated from body length with a log–log regression equation based on a large sample of previously measured mysids ($Y = 0.012X^{2.51}$, where Y is stomach volume (cubic millimetres) and X is carapace length (millimetres); $n = 70$, $r^2 = 0.94$), and the volume of material in the stomach could then be approximated as the fractional fullness of the stomach times the stomach capacity.

Experiment 3 was designed to assess the rate of passage of material through the guts of mysids that were not feeding. Four mysids were held overnight at 10°C in a 3-L tray of lake water with a dense mixture of plankton (mostly *Diaptomus* copepodids). The next day, the mysids were transferred to a small (14 × 8 × 5 cm) polyethylene box suspended in a larger (3.5-L) tray of filtered lake water. The bottom of the box, consisting of coarse (3.5-mm) plastic mesh, was several centimetres above the tray bottom. Fecal pellets produced by the four mysids in the box sank through the mesh to the bottom of the tray, where they could be collected with a pipet. The mysids, which apparently had full stomachs at the beginning of the trial, were held in this apparatus for >3 d, during which fecal pellets were collected nine times. The volume of feces was determined microscopically, and at the end of the experiment, the stomachs of the four mysids were dissected out and their degree of fullness estimated visually.

Results

The results of Experiments 1 and 2 are shown in Table 2. For 10 of the 31 mysids used in these trials, the experiment was

TABLE 2. Results of Experiments 1 and 2, showing passage times and ingestion and egestion rates for mysids fed red copepods and *Daphnia* sequentially.

Mysid No.	Copepod passage time (h)	No. of *Daphnia* in jar	*Daphnia* passage time (h)	Ingestion rate (*Daphnia*·h^{-1})	Egestion rate (mm^3 feces·h^{-1})
Experiment 1					
1	4.0	2	5.4	0.96	0.0293
2	8.0	5	6.8	1.24	0.0221
3	5.7	5	>7.6	1.21	0.0185
4	2.6	5	2.6	2.89	0.0843
5	1.8	5	2.6	1.50	0.0357
6	4.1	2	5.9	1.16	0.0242
7	4.8	5	>6.5	0.92	0.0148
8	4.3	2	6.9	1.10	0.0190
9	3.2	2	>6.5	0.82	0.0312
10	>10.0	2	>7.8	0.20	0
Experiment 2					
1	2.9	1	>8.1	0.23	0.0035
2	5.0	1	>11.0	0.40	0.0051
3	1.8	1	9.4	0.56	0.0147
4	2.9	1	6.6	0.63	0.0072
5	1.8	1	8.0	0.23	0.0057
6	>13.2	1	>10.3	0.08	0.0063
7	2.9	1	>4.5	0.16	0.0009
8	2.1	5	5.2	1.67	0.0191
9	>13.1	5	>11.0	0.31	0
10	1.0	5	2.1	0.60	0.0137
11	0.9	5	4.2	1.49	0.0211
12	0.8	5	7.9	1.85	0.0338
13	0.9	5	—	0	0.0063
14	0.9	5	7.3	0.75	0.0071
15	10.4	10	9.6	0.80	0.0105
16	0.9	10	1.1	1.98	0.0253
17	1.9	10	2.2	2.96	0.0479
18	>13.2	10	—	0	0
19	0.9	10	3.2	1.93	0.0351
20	2.1	10	6.7	1.46	0.0108
21	8.9	10	6.5	1.35	0.0108

terminated before both the copepod and the first *Daphnia* remains could be passed through the guts; the passage times recorded in these cases are, therefore, underestimates (Table 2). The times required for individuals to pass daphnid remains were often greater than the corresponding passage times for copepod meals; this tendency was significant for Experiment 2 (Wilcoxon signed rank test on mysids for which unambiguous data are available: $n = 14$, two-tailed $P < 0.01$) but not for Experiment 1 ($n = 6$, $P > 0.10$). All mysids consumed some copepod material at the beginning of the trials, but two mysids, including one (No. 13) that molted during Experiment 2, ate no *Daphnia*, so that daphnid passage times could not be measured.

There is a great deal of variability in the passage times reported in Table 2, from <1 to >13 h. Daphnid passage times were negatively correlated with average ingestion rates on *Daphnia*, calculated on a whole-experiment basis (Fig. 1): when nonparametric correlation analyses were run on unambiguous data from each experiment, a significant relationship was found for Experiment 2 (Spearman rank correlation coefficient $r_s = -0.56$, $n = 14$, two-tailed $P < 0.05$) but not for Experiment 1 ($r_s = -0.58$, $n = 6$, $P > 0.2$). If the two experiments are treated as independent tests of the same hypothesis, with the above probabilities combined according to Fisher's technique (described in Sokal and Rohlf 1981, p. 780), a correlation between passage time and ingestion rate is supported ($P < 0.05$).

Even though some of the mysids produced red fecal pellets soon after the start of the trials, when feeding on *Daphnia* had just begun (Table 2), copepod passage times were still negatively related to ingestion rates on *Daphnia* averaged over the whole of Experiment 2 (with the three indeterminate values ranked equally, $r_s = -0.47$, $n = 21$, two-tailed $P < 0.05$). The trend was similar, though not statistically significant, for Experiment 1 ($r_s = -0.39$, $n = 10$, $P > 0.2$). An aggregate probability for the two experiments (Sokal and Rohlf 1981) is statistically significant ($P < 0.05$).

The inverse relationship between gut residence time and feeding rate stems from a tendency for egestion rate to increase with the number of *Daphnia* consumed, unbalanced by any significant increase in stomach content (Fig. 2). The correlation between egestion and ingestion rates was significant for Experiment 2 ($r_s = 0.88$, $n = 21$, two-tailed $P < 0.005$) but not for Experiment 1 ($r_s = 0.56$, $n = 10$, $0.05 < P < 0.10$); the correlation between stomach content and ingestion rate was not significant in either case (Experiment 1: $r_s = 0.04$, $P > 0.9$; Experiment 2: $r_s = 0.30$, $P > 0.15$).

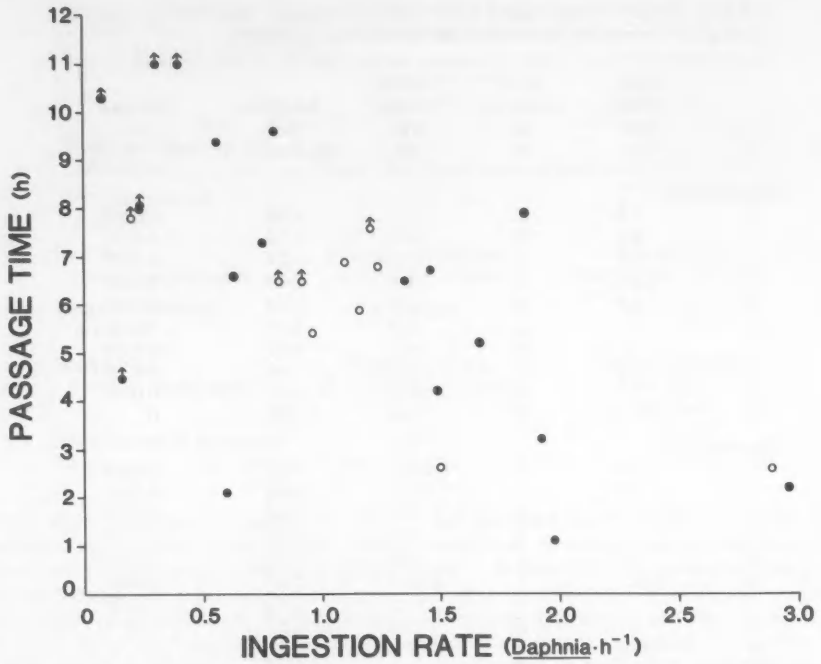

FIG. 1. Passage times of daphnid remains as a function of ingestion rate for *Neomysis* in Experiment 1 (open symbols) and Experiment 2 (closed symbols). Symbols with arrows indicate underestimates due to failure of mysids to egest daphnid remains. Ingestion rates on *Daphnia* are averages over the whole experiment.

The decrease in collective stomach fullness of the four mysids held in the food-free environment of Experiment 3 is shown in Fig. 3. These data represent average defecation rates calculated from the cumulative fecal pellet production of all four mysids, since the individuals were not isolated from one another. The starting volume of food per mysid stomach (0.665 mm^3) was obtained by summing the total fecal pellet production per mysid (0.385 mm^3) and the average final volume of food per stomach determined visually (0.280 mm^3). Under the assumption that the measured stomach capacities of the mysids were completely taken up by food at the beginning of the experiment, the expected initial volume of food per mysid (0.681 mm^3) is very close to the value predicted by summing fecal pellet production and final stomach content (0.665 mm^3), in spite of suggestions from other experiments that digestion and compaction may substantially reduce the volume of material passing through the gut (P. A. Murtaugh, unpubl. data). Apparently, prolonged digestion substantially reduced the volume of material retained in the stomachs of mysids in this experiment.

Since the mysids did not feed during this 3-d experiment, the egestion of material from the gut can be described with a simple exponential model (Elliott 1972; Thorpe 1977):

$$(1) \quad S_t = S_0 e^{-kt}$$

where S_t and S_0 are the amounts of material in the gut at time t and time 0, respectively, and k is the evacuation rate constant in units of reciprocal time and the magnitude of the slope of a plot of ln S vs. t. The regression line through all 10 points in Fig. 3b, corresponding to $k = 0.0101 \cdot h^{-1}$, is not a particularly good fit; as shown with broken lines through two subsets of the data, the evacuation rate constant in the first day or so after separation from food ($0.0198 \cdot h^{-1}$) is higher than that in the last 2 d of the experiment ($0.0037 \cdot h^{-1}$). The mysids apparently reduce their gut clearance rate in response to the absence of food, and significant amounts of material are retained in the guts even after 3 d.

For comparison with the data from Experiments 1 and 2, evacuation rate constants can be converted to median passage times, i.e. the time for 50% of the stomach content to be egested ($0.693/k$) (Elliott 1972). The solid line in Fig. 3b yields a median passage time of 69 h; the two broken lines, 35 and 187 h. Median passage times for individual mysids in the experiment, based on initial and final stomach fullnesses, were 18, 34, 105, and 151 h. All these times are much longer than those observed for the actively feeding animals in Experiments 1 and 2.

Discussion

Experiments 1 and 2 show that there is a great deal of variability in gut residence time for *N. mercedis* and that ingestion rate explains at least some of this variability: passage times decrease as the rate of feeding increases (Fig. 1). The mysids in these experiments maintained a relatively constant stomach fullness, while egestion rate tended to increase with ingestion rate, leading to shorter passage times (Fig. 2). The starved mysids in Experiment 3, under conditions of temperature and confinement similar to those in the other trials, retained material in their guts for >3 d, with a median passage time of 69 h; the reduction in evacuation rate of these animals after

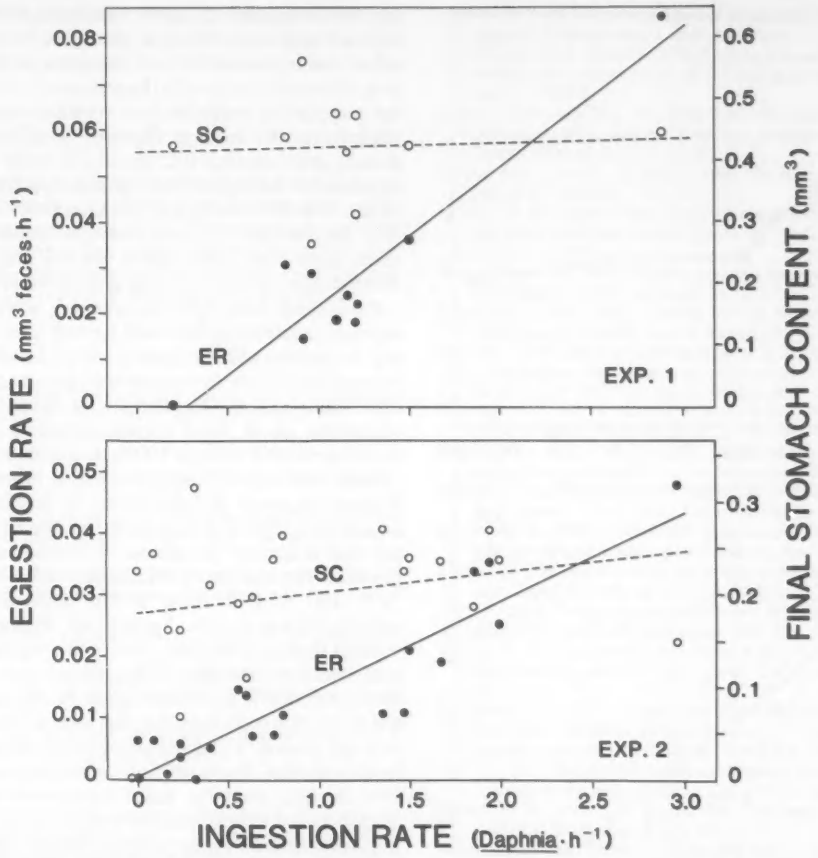

FIG. 2. Egestion rate (closed circles, solid lines labeled ER) and average stomach content (open circles, broken lines labeled SC) vs. average ingestion rate on *Daphnia* for *Neomysis* in Experiments 1 and 2. Lines are least-squares regressions.

about a day of starvation (Fig. 3b) is further evidence that the rate of processing is sensitive to food availability.

Another potential source of variability in gut residence time, not tested for here, is temperature. A decrease in passage time with increasing temperature has been reported for many aquatic animals, including amphipods (Marchant and Hynes 1981; Welton et al. 1983), *Chaoborus* larvae (Fedorenko 1975), and fishes (e.g. Persson 1982). Egestion rates of *N. mercedis* increase with temperature (Johnston and Lasenby 1982; P. A. Murtaugh, unpubl. data), but since gut residence time depends on ingestion rate and average stomach content as well as egestion rate, this is not conclusive evidence that residence time is temperature dependent.

There are suggestions in the literature that mysid evacuation rates may generally be quite variable. Stålberg (1934) reported that *Mysis relicta* passed carmine particles in 0.5–5.5 h, and he suspected that gut retention time varied with hunger and food quality and quantity. Rybock (1978) used a regression between egestion and ingestion rates for *M. relicta* to infer feeding rates from stomach contents but did not report gut passage times; he did observe that copepod mandibles may remain in the stomach for more than 10 h after the cessation of feeding. Molloy (1958) observed passage times of 0.5–1.5 h for *Neomysis integer* feeding on carmine but reported widely varying retention times in other cases: remains of copepods eaten by *Mesopodopsis slabberi* were sometimes eliminated within 2 min of ingestion (p. 104), while mysids of several species often retained material in the gut 5–7 d after being deprived of all food (p. 76, 96). Molloy removed feces from bowls containing starved mysids at frequent intervals but reported observing a specimen of *Neomysis* ingest a fecal string as it was extruded from the anus (p. 100). If common, this sort of instantaneous coprophagy would very much complicate the determination of gut passage times, as well as any attempt to infer feeding rate from stomach fullness.

To what extent my findings can be generalized to other organisms is unclear. Using ^{14}C-labeled food, Arashkevich (1977) found a decrease in digestion time with increasing food concentration for two marine copepods, though in other work with copepods defecation rate was independent of food availability (Arashkevich and Tseytlin 1978). Welton et al. (1983) documented substantial variability in the gut passage time of the amphipod *Gammarus pulex*, including a strong temperature dependence. Marchant and Hynes (1981) noted a similar effect of temperature in *G. pseudolimnaeus*, as well as a slowing of gut clearance after 1.5 h of starvation (cf. my Fig. 3). The

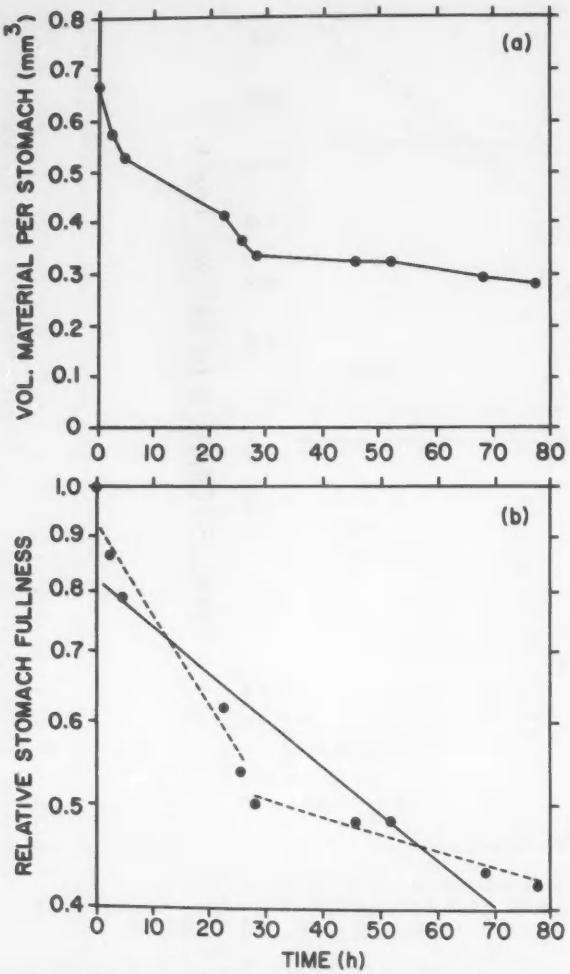

FIG. 3. Loss of material from stomachs of four mysids in food-free environment of Experiment 3. (a) Average amount of material per stomach, with egestion measured as the volume of feces collected from the tray bottom at the times indicated. Initial volume taken as sum of fecal pellet production and the visual estimate of the amount of material remaining in the stomachs at the end of the experiment. (b) Semi-log plot of the change in relative stomach fullness, with least-squares regression lines fit to all the points (solid line: $\ln Y = -0.205 - 0.0101X$) or to subsets of the data (broken lines: first 5 points, $\ln Y = -0.080 - 0.0198X$; last 5 points, $\ln Y = -0.573 - 0.0037X$). Magnitudes of slopes of lines are estimates of the evacuation rate constant k. Mysid lengths were 13.2–14.9 mm.

feeding methods of euphausiids have been compared with those of the mysid *Hemimysis lamornae* (Mauchline and Fisher 1969), but data on euphausiid gut clearance are scarce: starved *Euphausia superba* retained material in their guts from a few hours to more than 7 d (Antezana et al. 1982), whereas Heyraud (1979), by a very indirect method, estimated transit times of 15–30 min for *Meganyctiphanes norvegica*. In experiments in which animals were fed zooplankton and then isolated in food-free containers, chaetognaths and ctenophores defecated their meals rather promptly: mean digestion times of 3.17 h (Feigenbaum 1979) and 1–2 h (Reeve 1980) have been reported for species of *Sagitta*, and the ctenophore *Mnemiopsis* digested single prey items in about 2 h (Reeve 1980). These values contrast with the tenacious retention of material in the guts of starved *N. mercedis* (Experiment 3), though there is still the potential for variability in evacuation rates of these marine planktivores: the average digestion time for *Sagitta elegans* feeding on copepods at 0°C was 10.2 h, much longer than times reported for chaetognaths at higher temperatures (Feigenbaum 1982). Variability in the gut residence times of fishes has been well documented, and environmental influences on passage times have been much better studied than in the case of invertebrates (review by Fänge and Grove 1979).

For animals with digestive dynamics similar to those of *N. mercedis*, attempts to infer field feeding rates from gut fullness will be problematic. A popular use of invertebrate stomach-fullness data is in the investigation of the circadian periodicity of euphausiid feeding. Occasionally, stomach fullness has been interpreted as an index of instantaneous feeding intensity (Ponomareva 1971; Roger 1975), though the change in stomach fullness with time would seem a more meaningful quantity. Without exception the assumption of constant gut residence time is made when diel changes in the amount of food in the gut are used to identify the periods of most active feeding (early literature summarized by Mauchline and Fisher 1969; Roger 1973; Hu 1978; Sameoto 1980). Since many euphausiids undergo extensive vertical migrations, authors often relate the inferred rhythm of feeding activity to temporal changes in the food densities encountered by the animals (e.g. Hu 1978; Sameoto 1980). If gut residence time is affected by temperature and by the rate of food intake, diel changes in stomach fullness may not present a completely accurate picture of the actual feeding rhythm. Euphausiids in cold, deep water might retain food in their stomachs much longer than actively feeding animals in the warmer surface waters. A small diel change in stomach fullness, rather than indicating continuous feeding (Roger 1973), might represent the effect of a variable gut residence time masking a real circadian feeding periodicity. Perhaps this influence of gut residence time, and differences among species in the extent to which it operates, explain why different authors have reached conflicting generalizations about the relationship between the strength of vertical migration and degree of feeding periodicity (Ponomareva 1971; Hu 1978 vs. Roger 1975).

Although the problems of translating gut contents into feeding rates may be formidable, useful information can nevertheless be obtained from field data on stomach fullness. At the very least, an increase in the amount of material in the gut over time gives a minimum estimate of ingestion rate, while a decrease provides a minimum estimate of egestion rate. More detailed inferences, about absolute feeding rates or spatial and temporal fluctuations of feeding intensity, require knowledge of gut residence times and their dependence on environmental conditions and feeding history. For some kinds of animals, such as chaetognaths and ctenophores, gut residence times may be relatively easy to predict. Finally, other kinds of information such as the composition of the diet (Sameoto 1980), the extent of digestion of prey remains (Purcell 1981), and the relative amounts of food in the stomach and intestine (Bowman and Bowman 1980) can give clues about the time and place that the material in the gut was ingested.

Probably not all predators show the remarkable flexibility in evacuation rate documented here for *N. mercedis*. But for any animal, gut fullness is affected by egestion rates as much as by

ingestion rates, and a clear understanding of the magnitude and variability of gut residence time should be prerequisite to any attempt to infer feeding intensity from the amount of food in the alimentary tract.

Acknowledgments

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Selective Exploitation of Capelin (*Mallotus villosus*) Eggs by Winter Flounder (*Pseudopleuronectes americanus*): Capelin Egg Mortality Rates, and Contribution of Egg Energy to the Annual Growth of Flounder[1]

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Frank, K. T., and W. C. Leggett. 1984. Selective exploitation of capelin (*Mallotus villosus*) eggs by winter flounder (*Pseudopleuronectes americanus*): capelin egg mortality rates, and contribution of egg energy to the annual growth of flounder. Can. J. Fish. Aquat. Sci. 41: 1294–1302.

The consequences of winter flounder (*Pseudopleuronectes americanus*) predation on capelin (*Mallotus villosus*) eggs, both in terms of the egg mortality and of the contribution of energy ingested to the annual growth of flounder, were investigated at Bryant's Cove, Conception Bay, Newfoundland. Flounder density in the nearshore area adjacent to the capelin spawning beach increased sharply in each of the three study years in response to the presence of spawning capelin and to the density of capelin eggs on the spawning beaches. Capelin eggs were the dominant item in the diet of winter flounder 14–34 cm TL, averaging 59% by weight. Flounder ⩾35 cm TL fed less heavily on capelin eggs (22% of food consumed by weight) but because of their larger size and ration were an important source of predation. Egg mortality due to flounder predation was estimated to average 5.0, 2.2, and 1.9% in 1978, 1979, and 1980, respectively. Egg mortality due to predation is believed to be significantly greater at deepwater demersal spawning sites and may have a significant impact on recruitment in demersal spawners. The average energy gain to winter flounder resulting from consumption of capelin eggs was estimated to contribute 23% of the annual growth of flounder in the region (approximate 95% confidence interval: 10–40%). Selective exploitation of capelin eggs by postspawning flounder may also contribute significantly to the rapid recovery of body condition following the extensive depletion of tissue resources due to winter fasting, gonad production, and spawning.

On a étudié les conséquences de la prédation de la plie rouge (*Pseudopleuronectes americanus*) sur les œufs de capelan (*Mallotus villosus*) en termes de la mortalité des œufs et de la contribution de l'énergie ingérée à la croissance annuelle de la plie dans l'anse Bryant (baie de la Conception, Terre-Neuve). Lors de chacune des trois années d'étude, la densité des plies dans la zone littorale adjacente à la frayère du capelan a nettement augmenté en réaction à la présence de capelans reproducteurs et à la densité des œufs sur les plages. Ceux-ci constituaient le principal élément (59 % du poids en moyenne) du régime des plies rouges mesurant de 14 à 34 cm LT. Les plies mesurant ⩾35 cm LT mangeaient moins d'œufs de capelan (22 % du poids total des aliments) mais, à cause de leur plus grande taille et de leur plus grande consommation, elles représentaient une importante source de prédation. En 1978, 1979 et 1980, on a estimé à 5,0, à 2,2 et à 1,9 % respectivement la mortalité moyenne des œufs par la prédation des plies. On croit que la mortalité des œufs par prédation est nettement plus élevée dans les frayères démersales en eaux profondes et qu'elle peut avoir une incidence significative sur le recrutement des géniteurs démersaux. On évalue le gain énergétique moyen résultant de la consommation d'œufs de capelan à 23 % de la croissance annuelle de la plie rouge dans la région (intervalle de confiance approximatif de 95 % : 10–40 %). L'exploitation sélective des œufs de capelan par les plies après la fraie peut aussi contribuer pour beaucoup au rétablissement rapide de leur condition corporelle, après l'intense utilisation des ressources tissulaires due au jeûne hivernal, à la production de gonades et à la fraie.

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Aggregations of predators in areas of high prey density generally involve mobile predators and relatively immobile prey (Sih 1984). The highly concentrated, contagious distribution of eggs on the spawning beds of demersal spawning fishes is known to attract high densities of predatory fishes and occasionally of diving ducks and invertebrates (Richards 1963; McKenzie 1964; Grosslein et al. 1980; Tibbo et al. 1963; Gjosaeter and Saetre 1974; Caddy and Iles 1973; Dragesund and Nakken 1973; Templeman 1965; Taylor 1964). This has led to the belief that eggs of demersal spawners experience high mortality due to predation, especially since

[1]Contribution to the program of GIROQ (Groupe interuniversitaire de recherches oceanographique du Québec.

defensive mechanisms such as parental care and inedible eggs are often lacking. Subtidal spawning Pacific herring (*Clupea pallasi*) incur levels of egg loss, attributable to bird predation, ranging from 30 to 55% (Taylor 1964). Saetre and Gjosaeter (1975) reported that 70% of the annual catch of haddock (*Melanogrammus aeglefinus*) contained capelin eggs. In the North Sea, the stomach of a "spawny" haddock typically contains at least the annual egg production of one herring (*Clupea harengus*) during the herring spawning season (Hempel 1971). Dragesund and Nakken (1973) also identified egg predation by haddock as the most important source of mortality of Norwegian spring spawning herring. They concluded that haddock could consume as much as 40% of the estimated annual herring spawn production. Tibbo et al. (1963) calculated that the winter flounder (*Pseudopleuronectes americanus*) population on the Chaleur Bay herring spawning bed consumed at least 5×10^9 herring eggs in a single meal.

Severe predatory losses may be one factor in the evolution of the very high fecundities generally found among demersal spawners. Beach spawning behavior exhibited by grunion (*Leuresthes* sp.), capelin (*Mallotus villosus*), and several Pacific smelts (e.g. *Hypomesus* sp., *Spirinchus* sp.), which results in their eggs being buried in the beach sediments (see reference in Frank and Leggett 1981a), may contribute to a reduction in mortality by making the eggs more difficult to obtain.

The intensity of egg predation on demersal spawners is an important problem from both an applied and theoretical perspective. At the population or community level, predators may act as important selective mortality agents on the evolution of prey reproductive systems. Knowledge of the predator–prey relationships between demersal spawners and their egg predators is also instrumental to the development of management strategies for commercially important species. Despite the considerable interest shown in this subject (reviewed in Hempel 1971; Dahlberg 1979; Hunter 1980; and numerous papers in Lasker and Sherman 1981), logistic difficulties involved in locating and studying predator–prey activity on offshore spawning beds (normally requiring the use of submersibles and/or SCUBA; Van Guelpen and Davis 1979; Bakke and Bjorke 1973; Grosslein et al. 1980; Caddy and Iles 1973; Pottle et al. 1981) have severely limited the number of quantitative estimates of its impact.

An equally important but little understood question is the contribution of the energy contained in demersal spawn to the annual energy budget and/or growth regimes of the predators. Once located, demersal egg beds offer a concentrated, energy-rich resource that is easily exploited. Although this has frequently been acknowledged, and the behavior of fishes in response to such seasonal food sources supports the argument, there have been few quantitative studies of its importance (Hempel 1971; Klein-MacPhee 1978).

In this study we examined the mortality imposed by winter flounder predation on the eggs of beach spawning capelin, and the contribution of capelin eggs to the annual energy budget of winter flounder.

Methods

In June and early July large schools of mature capelin migrate from their overwintering areas to spawn on exposed beaches. The adhesive eggs become mixed with the intertidal sediments from the high to low tide zones. Densities can exceed 150 eggs/cm^3. Time to hatching varies from 2 to 4 wk depending on temperatures in the gravel (Frank and Leggett 1981a, 1981b). Winter flounder respond to the presence of spawning capelin by forming dense aggregations in the nearshore areas adjacent to the beaches (Kennedy and Steele 1971; Van Guelpen and Davis 1979) where they feed heavily on both eggs and adults.

We examined the predator–prey interaction between capelin and winter flounder during the summers of 1978, 1979, and 1980. The work was conducted at Bryant's Cove (47°40′36″N, 53°22′24″W) a major spawning site for capelin in Conception Bay, Newfoundland. Full details of the study site are provided in Frank and Leggett (1981a, 1981b).

To quantify the abundance of winter flounder in the nearshore area of the cove, we counted the number of fish located within 1–6 m on either side of three 90-m transects oriented parallel to the beach at distances of 0, 15, and 30 m from the low tide zone. Counts were made between 09:00 and 11:00 three times weekly using SCUBA. Turbidity caused differences in the visible area along each transect. Visibility was therefore determined by the divers prior to each survey of the transects. The number of flounder per 100 m^2 along the transect was expanded to give an estimate of the total number of flounder within 35 m of the low tide zone.

Abundance of capelin eggs on the beach from spawning until the termination of hatching was estimated by coring the beach at high tide, mid-tide, and low tide zones at each of three egg coring sites (details of the egg sampling method are given in Frank and Leggett 1981b). The average number of eggs per cubic centimetre in the low, mid-, and high tide cores on each date were integrated over the respective areas to provide twice weekly estimates of egg numbers on the beach throughout the period of study.

Capelin spawning in the nearshore zone, away from the beach, occasionally occurs after the principal beach spawning period (Templeman 1948). To assess off-beach egg deposition we attached three weighted, artificial substrates (0.5 × 0.5 m) made to resemble the sediment of the surrounding substrate to each of the 0-, 15-, and 30-m transect lines. The number of eggs attached to each artificial substrate was counted during each SCUBA survey. All eggs were removed from the artificial substrates after each count.

The diet of winter flounder throughout the spawning and incubation period was determined from random samples of 8 to 16 fish collected weekly using a hand spear during the SCUBA survey. The complete digestive tract was removed and preserved in 4% formalin immediately after capture. Length and weight of each fish was recorded prior to dissection. Gut contents were sorted into six categories: capelin eggs (live and dead), adult capelin, plant material, molluscs, annelids, and crustaceans (primarily amphipods). Each food type was dried to constant weight (24 h at 70°C), removed to a desiccator, and weighed to an accuracy of 10 μg.

Daily ration of winter flounder was estimated from data on diel feeding periodicity. Feeding periodicity was determined by sampling 20 fish every 4 h over a 30-h period on 23–24 June 1980. All fish sampled were treated as described for diet analysis. We calculated daily ration using the method of Nakashima and Leggett (1978). This method requires no assumptions concerning evacuation rates or constancy of feeding as do more complex models (Thorpe 1977; Eggers 1977; Elliot and Persson 1978) and yields estimates that do not

differ significantly from those derived from these more complex models (Nakashima and Leggett 1978; Nakashima 1979).

The reliability of this estimate of daily ration was evaluated by determining the rate of stomach evacuation. Evacuation rates were determined by force-feeding flounder (size range 27.5–50 cm) with capelin eggs at a ration of 5% of their body weight. All flounder were starved for 48 h prior to feeding by holding them in a 2 × 2 × 1 m cage moored approximately 200 m from the spawning beach. Fish were anesthetized with MS 222 immediately prior to handling for feeding. The meal (capelin eggs) was administered from a large-bore syringe fitted with Tygon tubing, which was inserted into the esophagus. Each fish was marked with a numbered Floy tag at the time of feeding. Immediately following force-feeding the fish were returned to the holding cage. Fish were then killed at 3- to 5-h intervals over a 24-h period. The exact time from feeding to sampling was determined by reference to the tag number. Two such experiments were conducted (1980: 7–8 July, water temperature = 7.8°C; 1981: 5–6 July, water temperature = 10.0°C).

The energy content of capelin eggs was determined by bomb calorimetry. All eggs used in these determinations were collected directly from the spawning beaches and were preserved in 4% formalin. Calorific determinations were made by the Crampton Nutrition Laboratory, Department of Animal Science, Macdonald College of McGill University. The gross energy content of capelin eggs was 5.957 kcal/g (dry weight) (1 kcal = 4.1868 J). One gram (dry weight) equates to approximately 16 000 eggs.

Results

Flounder and Egg Density

During the spawning and egg incubation period the density of winter flounder was consistently greatest at the 0-m transect (1978, 57%; 1979, 58%; 1980, 68%) and declined progressively with distance from the beach (Fig. 1). In both 1978 and 1979, maximum winter flounder densities coincided with the presence of large numbers of spawning or spent capelin near the spawning beach. In 1980, maximum densities closely tracked the density of capelin eggs on the spawning beach ($r^2 = 0.79$, $p < 0.01$; Fig. 1). Flounder density also exhibited a significant positive correlation with egg density in 1978 ($r^2 = 0.59$, $p < 0.01$) but this relationship was not apparent in 1979 (Fig. 1). The main period of capelin spawning in 1979 occurred 10–14 d earlier than in 1978 and 1980 and was more concentrated in time (Fig. 1). The maximum flounder abundance in 1979 was associated with a large concentration of predominantly male capelin, which occurred in the nearshore area between 12 and 26 July. The absence of an increase in capelin egg densities on the beach at this time indicates that spawning, if it occurred, was minimal. This large peak in flounder abundance at a time when no eggs were deposited explains, in part, the absence of a relationship between flounder and egg densities in 1979.

The mean and range of sizes of winter flounder sampled was similar between years (1978, 32 cm, SD = 9.3; 1979, 28 cm, SD = 8.5; 1980, 34 cm, SD = 8.1). Between 65 and 75% of the fish sampled had lengths within ±1SD of the mean in each of the three years (Fig. 2). Kennedy and Steele (1971) reported that 50% of winter flounder females and males in the Conception Bay region are sexually mature at 25 and 21 cm, respectively. In our survey, >70% of the flounder sampled were >25 cm TL

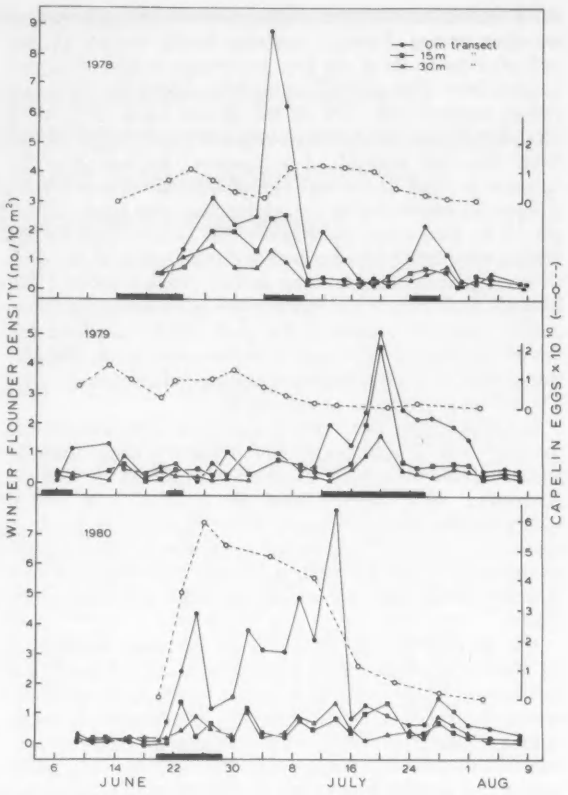

FIG. 1. Density of winter flounder adjacent to the capelin spawning beach during the June–August study period. Shading along the time scale indicates periods of high concentrations of spawning or spent capelin in the nearshore zone. Total egg deposition on the beach is also shown.

(Fig. 2). This suggests that the majority of the flounder in our samples were mature individuals.

The total number of capelin eggs on the spawning beach was similar in 1978 and 1979, reaching peaks of 12 × 10^9 to 15 × 10^9 eggs immediately after spawning. Peak egg abundance was approximately sixfold greater in 1980 both because of larger numbers of spawning capelin and a concentration of spawning activity into a single period (Fig. 1).

Diet of Winter Flounder and Daily Ration

The diet of winter flounder during the period of study was dominated by adult capelin and capelin eggs (1978, 60%; 1979, 70% by weight).

The proportion of eggs in the diet of flounder in the length range 14–34 cm TL exceeded that of all other food types, averaging 59% (dry weight). By comparison, the proportion of eggs in the diet of flounder ≥35 cm was lower, averaging 22%. The diet of these larger fish contained a significantly greater proportion of adult capelin, averaging 59% by weight (Fig. 3). The maximum number of eggs ingested by these larger flounder in a single meal ranged from 20 000 to 30 000. The average

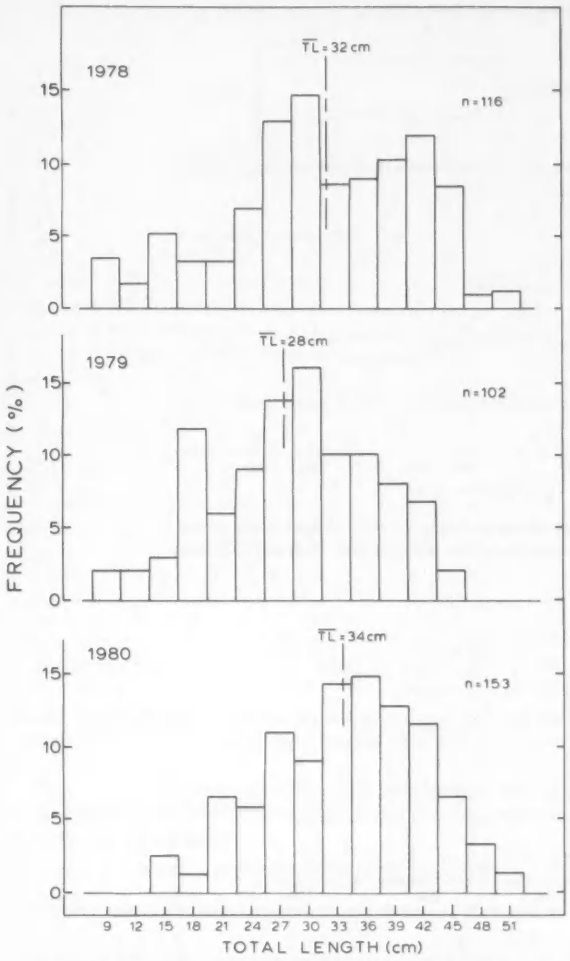

FIG. 2. Length–frequency distributions for winter flounder derived from the weekly field collections during the June–August study period in 1978 and 1979, and from the feeding periodicity study conducted in June 1980. Each length-class encompasses a 3-cm length interval.

composition of the diet of winter flounder over the total size range sampled (14–46 cm) was capelin eggs, 46%; adult capelin, 22%; crustaceans, 7%; annelids and molluscs, 12%; plant material, 13%.

In 1978 and 1979, temporal changes in the proportion of eggs and adult capelin in the diet were consistent with changes in the abundance of capelin eggs on the beach and spawning or spent capelin in the nearshore (Fig. 4). Feeding intensity (expressed as dry weight of all food items in stomach per dry body weight) also paralleled these changes in food availability, reaching maxima of 1.5 and 1.3% during the two capelin beach spawning events in 1978. In 1979, maxima of 1.3 and 1.5% occurred during a period of peak egg abundance on the beach and during a large concentration of spent capelin near the beach (Fig. 4). In 1978, the final peak in egg abundance in the diet of flounder coincided with off-beach spawning at depths of 2–3 m between the 0- and 15-m transect lines. Although this spawning was not reflected in the egg abundances on the beach it was detected on artificial substrates located primarily along the 0-m transect line (Fig. 5). In 1979, similar but less intensive off-beach spawning occurred between 12 and 26 July. The proportion of eggs and adult capelin in the diet of flounder increased significantly during this period (Fig. 5). Between 4 and 9 July 1980, off-beach spawning similar in magnitude to that recorded in 1979 was again observed (Fig. 5). In direct contrast with the two previous years flounder densities were high prior to and following this spawning event, which occurred while egg densities on the beach were still high.

Flounder exhibited a single distinct peak in gut fullness between 11:00 and 15:00 (Fig. 6). This is consistent with previous reports that flounder are visual feeders (Pearcy 1962; Olla et al. 1969). Capelin eggs comprised between 66 and 98% (dry weight) of the diet during the 30-h interval studied. The diet composition during this interval was similar to that observed in 26- to 34-cm flounder during 1978 and 1979 (Fig. 3).

Daily ration (dry weight) derived using the method of Nakashima and Leggett (1978) was estimated to be 3.92% of the dry body weight (95% confidence interval 3.16–4.68).

The gut evacuation rate of force-fed winter flounder is described by the equation $Y = 0.138 + 0.1393X$ ($r^2 = 0.93$, $n = 9$, $p < 0.01$) where Y = (g (dry weight) digested/g dry body weight) × 100 and X = time since force-feeding in hours (Fig. 7). There was no significant difference in the rate in the two years in which the determinations were made.

The daily assimilation rate, based on the force-feeding data, was 3.48% of dry body weight. This is not significantly different from the daily ration estimate derived above. The slightly lower average value derived from force-feeding is not unexpected given the handling stress involved.

Capelin Egg Mortality due to Predation by Flounder

Total egg ingestion by flounder over the spawning and incubation period was estimated using the equation

$$(1) \quad I_T = (\bar{B} \cdot \bar{R} \cdot \bar{T})C$$

where I_T = total egg ingestion (grams dry weight), $\bar{B}$ = average consumer biomass (grams dry weight), $\bar{R}$ = daily ration (grams dry weight per day), T = average number of days from first spawning to completion of hatching (40 d), and C = average proportion of capelin eggs in the diet (based on data from 1978 and 1979 contained in Figure 3) = 0.46.

Three estimates of I_T were derived for each year using the mean ($\bar{R}$), and upper and lower 95% confidence interval for $\bar{R}$ as derived using the method of Nakashima and Leggett (1978).

Egg mortality was estimated as

$$(2) \quad M = (I_T \cdot N / D_T)100$$

where M = capelin egg mortality (percent), I_T = total egg ingestion (grams dry weight), N = number of capelin eggs per gram dry weight = 16 000, and D_T = total egg deposition = Σ number of eggs on the beach immediately following spawning events.

Egg consumption by winter flounder during the spawning and incubation periods of 1978, 1979, and 1980 ranged from 1.6 to 5.9% of the total egg deposition (Table 1).

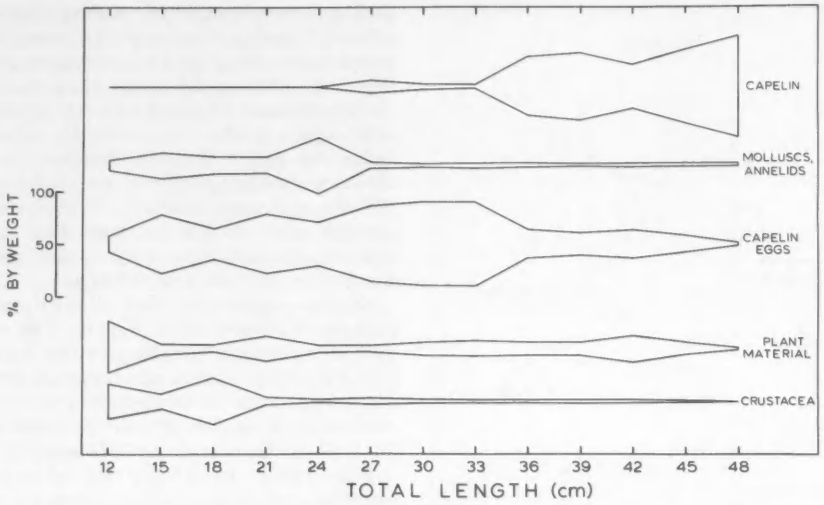

FIG. 3. Diet composition of 13 size-classes of winter flounder during the June–August study period. Percentages for each food type were weighted within each size-class, and data from 1978 and 1979 were combined for the analysis.

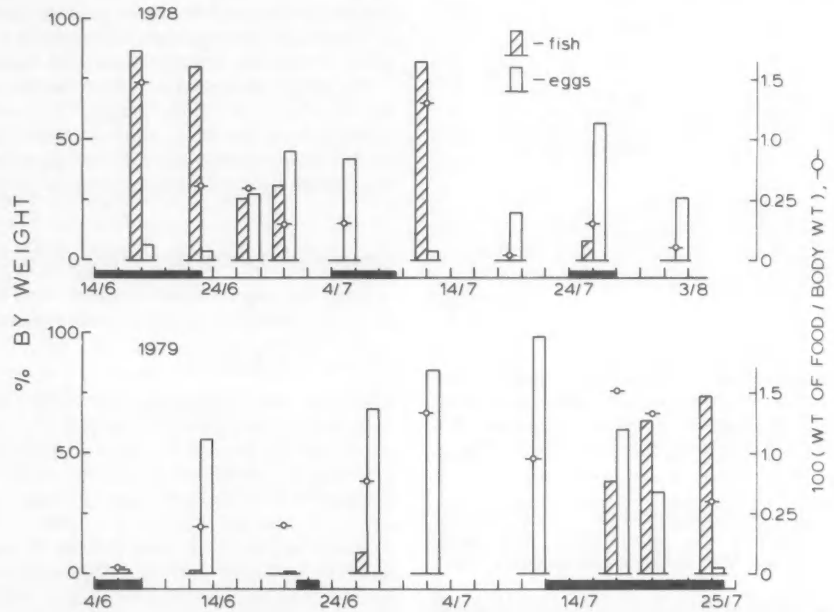

FIG. 4. Temporal changes in feeding intensity and the percentage of capelin eggs and adult capelin in the diet of winter flounder weighted over all size-classes. Shading along the time axis indicates periods of concentration of spawning or spent capelin in the nearshore.

Contribution of Capelin Eggs to Annual Energy Budget of Flounder

We estimated the contribution of energy derived from the ingestion of capelin eggs to the annual growth of flounder by relating calories consumed to previously published estimates of the growth rate of winter flounder (grams per day) sustained at various ration levels (Tyler and Dunn 1976). Average water temperatures at our Newfoundland study site (1978, 7.9°C; 1979, 6.9°C; 1980, 6.5°C) were similar to those used in Tyler and Dunn experiments (7.5 ± 0.5°C). Because daily ration is calculated as a function of body weight, we based our calculation on the modal size (30 cm) of fish in our 1978 and 1979 collections (Fig. 2).

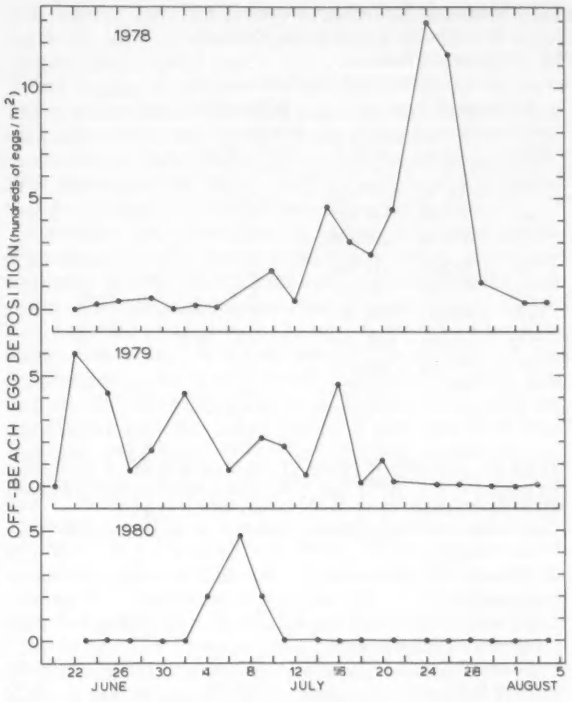

FIG. 5. Daily changes in the number of capelin eggs deposited on artificial substrates positioned on the bottom of the nearshore zone.

Daily ration estimates (3.92%, 95% confidence interval 3.16–4.68%) were converted to kilocalories per day and used as input into the equation

(3) $G = -1.0608 + 0.21785R$ ($r = 0.85$, $p < 0.01$)

where G = daily growth rate (grams per day) and R = ration (kilocalories per fish per day). This equation was derived from table 1 of Tyler and Dunn (1976) and is based on all fish 37–40 cm TL experiencing a positive weight change when fed a diet of clams and beef liver.

Weight gain from egg energy consumed during the period of feeding on capelin eggs was calculated as

(4) $W = G_c \cdot T$

where W = total weight gain (grams), G_c = growth rate (from eq. 3) adjusted for $C = 0.46$, and T = average number of days from spawning to completion of hatching = 40 d.

The annual growth (W_A) of 30-cm winter flounder in the Conception Bay area was reported by Kennedy and Steele (1971) to be 2.0 cm for males and 5.0 cm for females. We assumed an average annual increase of 3.0 cm and converted this to weight gain using the length–weight relationship

(5) $Y(\text{wet wt, g}) = 0.0248\text{TL}^{2.8277}$ ($r^2 = 0.97$, $n = 153$)

which we derived for winter flounder sampled during our study. The estimated average annual weight gain for 30-cm flounder was 115 g.

The contribution of egg energy to annual growth in flounder was 23%. Applying the upper and lower 95% confidence limits for daily ration to these calculations yielded estimates ranging from 13 to 26% of annual growth (Table 2).

To assess the influence of known variance in the variables used to estimate the contribution of capelin eggs to annual growth in flounder, we performed an error analysis in which the values of the input variables were caused to vary simultaneously by selecting them at random assuming a normal distribution. On each iteration all input values were chosen from the specified distribution according to their probability of occurrence in that distribution. The total time during which eggs were available to flounder was varied between 30 and 50 d ($\bar{x}$ = 40 d). Daily ration was varied between 3.16 and 4.68% dry weight ($\bar{x}$ = 3.92%). The proportion of eggs in the diet was varied between 10 and 90% ($\bar{x}$ = 46%) and the annual growth increment was varied between 2.0 and 5.0 cm ($\bar{x}$ = 3.0 cm). The distribution of

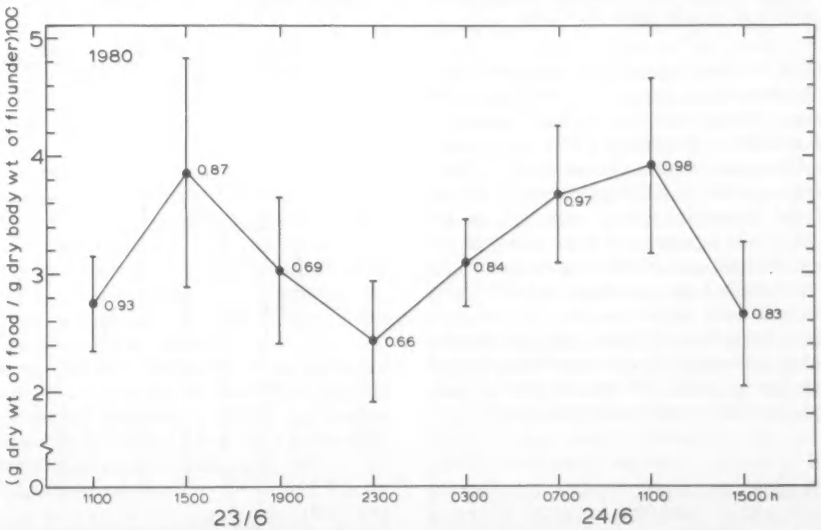

FIG. 6. Feeding periodicity of winter flounder. Each value is the mean of up to 20 fish collected at 4-h intervals in the nearshore zone adjacent to the capelin spawning beach. Values next to mean show proportion by weight of capelin eggs in diet. Vertical bars indicate 95% confidence intervals.

TABLE 1. Summary of parameter values used in Eq. 1 to estimate capelin egg mortality due to predation by winter flounder. Symbols are defined in the text (see results on capelin egg mortality). L_1 = lower 95% confidence interval; L_2 = upper 95% confidence interval.

	Egg deposition D_T	Biomass (g) $\bar{B}$	Ration R	Ingestion (g) Daily	Ingestion (g) I_T	kcal/T	% Mortality $100(I_T \cdot N)/D_T$
1978	22.6×10^9	97 239	$\bar{R}$	1753	70 137	417 805	5.0
			L_1	1413	56 539	336 801	4.0
			L_2	2093	83 734	498 806	5.9
1979	27.6×10^9	53 522	$\bar{R}$	965	38 604	229 966	2.2
			L_1	778	31 120	185 381	1.8
			L_2	1152	46 089	274 551	2.7
1980	58.9×10^9	98 549	$\bar{R}$	1777	71 081	423 432	1.9
			L_1	1433	57 300	341 338	1.6
			L_2	2122	84 863	505 526	2.3

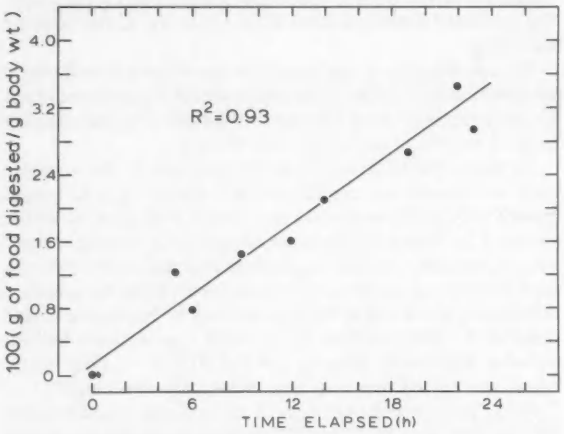

FIG. 7. Relationship between the proportion of capelin eggs (5% of body weight) digested and the time since force-feeding for winter flounder. Each data point represents the mean of 3–5 fish.

TABLE 2. Summary of parameter values used in Eq. 4 to calculate the contribution of capelin eggs to the annual energy budget of a 30-cm TL winter flounder. Symbols are defined in the results.

Ration	$\bar{R}$	L_1	L_2
Ingestion (g/d)			
Dry wt	2.9	2.4	3.5
Equivalent energy (kcal/d)			
$c = 1.0$	17.28	14.30	20.85
$c = 0.46$	7.95	6.58	9.59
Growth (when $c = 0.46$)			
G (g/d)	0.67	0.37	1.03
W (g)	26.8	14.8	41.2
If assume annual weight gain $(W_A) = 115$ g			
$\frac{100W}{W_A}$ (%)	23	13	36

the expected contribution of capelin eggs to the annual growth of winter flounder based on 1000 iterations is given in Fig. 8. The analysis indicates that the probability of this contribution falling between 10 and 40% (the approximate 95% confidence interval as estimated from known variance in daily ration alone) is 90%. The average annual contribution, derived by log transformation of the output to achieve normality in the distribution, was 24%. The similarity of this value with the mean calculated from the static analysis was expected, since the specified normal distributions for the input variables were centered on the observed mean values.

We consider these to be minimum estimates because flounder feeding on energy-rich eggs should have a higher net conversion efficiency (K_2) than the value of 24% reported by Tyler and Dunn (1976) for flounder fed on clams and beef liver.

Discussion

This study and those by Tibbo et al. (1963), Kennedy and Steele (1971), Caddy and Iles (1973), Van Guelpen and Davis (1979), and Pottle et al. (1981) indicate that winter flounder respond rapidly to and form large aggregations ($\geq 1/m^2$) at energy-rich, demersal spawning sites. The sensory basis for

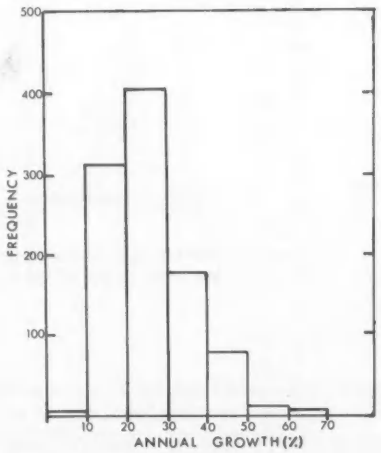

FIG. 8. Estimated contribution of capelin eggs to the annual growth of winter flounder derived from 1000 iterations of a model in which the values of the input variables were simultaneously selected at random from specified distributions based on observed variance.

such behavior was elucidated by Sutterlin (1975) in field trials, where the scent of natural baits and pure compounds were released from a point source that successfully attracted and elicited feeding behavior in winter flounder. The close proximity of winter flounder spawning sites to those of capelin (Frank and Leggett 1983) and a tendency for winter flounder to form locally discrete and stationary populations in such areas (Lobell 1939; Perlmutter 1947; Dickie and McCracken 1955) suggest a strong coupling of the life histories of the two species.

We found that winter flounder exhibit substantial energy dependence on the annual egg production of capelin, supporting an average of 23% of their annual somatic growth from this food source. While this contribution to somatic growth is substantial, it is important to emphasize that annual growth in sexually mature individuals, which characterize the majority of the flounder considered here, has two components: somatic and reproductive. The relative energy allocation to somatic versus reproductive growth is not known for winter flounder in situ. However, MacKinnon (1972) has provided such estimates for American plaice (*Hippoglossoides platessoides*) in Nova Scotia. His data indicate that energy for somatic growth is approximately 56% of the total annual energy allocation to growth (soma + reproductive products). If the energy allocation to somatic versus reproductive growth in mature flounder is similar, the contribution of capelin eggs to the *total* annual growth of mature flounder would range from approximately 6 to 22%. In immature fishes, where all surplus energy is allocated to somatic growth, the contribution of eggs to somatic growth is likely to approximate 10–40%. These are, of course, average values. The contribution of egg energy to the annual somatic growth of individual fish will vary depending on maturity, size-related differences in diet (Fig. 2), and the duration of their feeding on eggs. The analysis does demonstrate, however, that the contribution of capelin eggs to the annual energy budget of flounder is substantial. This helps to explain the precise coupling of the arrival of large numbers of flounder in the nearshore areas of eastern Newfoundland coincident with the onset of capelin spawning. It also explains the high densities of predators found at the spawning sites of other demersal spawning fishes.

Yearly variation in capelin egg abundance is high (spawner biomass varied 12.5× during 1972–80 (Carscadden and Miller 1981; see also Fig. 1)). This large variance in energy availability may significantly influence annual growth in those populations of winter flounder that systematically exploit capelin eggs. Furthermore, since winter flounder do not feed during the winter, and draw heavily on stored energy reserves to support gonad production prior to spawning in May–June (MacKinnon 1972; Roff 1982), the rate at which mature fish regain body condition, and perhaps even their survival, may be significantly influenced by their ability to detect and exploit capelin spawn. The preponderance of sexually mature individuals in the population we studied (Fig. 2) supports this view.

Our estimates of egg mortality attributable to predation are lower than those previously reported for herring and capelin spawning in offshore areas. For example, herring egg loss due to predation was estimated to exceed 7% in the Chaleur Bay (Northumberland Strait) (Tibbo et al. 1963), 15–40% in the Norwegian Sea (Dragesund and Nakken 1973), and 8% on Georges Bank (Caddy and Iles 1973). Templeman (1965) and Saetre and Gjosaeter (1975) considered capelin egg consumption to be "significant" at deepwater spawning sites (40–150 m).

The greater abundance of known egg predators (see the introduction) such as haddock, cod (*Gadus morhua*), red hake (*Urophycis chuss*), dogfish (*Squalus acanthias*), lumpfish (*Cyclopterus lumpus*), and skates (*Raja* sp.) at offshore spawning sites, the considerably larger body size of these offshore predators relative to the flounders we studied, and the prolonged hatching times that result from the lower incubation temperatures typical at deepwater spawning sites suggest that published estimates of offshore egg predation are at best conservative. The potential effect of increasing predator density and the duration of egg availability (development time) on egg mortality rates is dramatically illustrated in Fig. 9. Beach spawning therefore appears to be an effective means of reducing egg predation due both to a reduction in egg accessibility to most large fishes, especially gadoids, and to the thermal acceleration of egg development rates, which reduces the time that eggs are susceptible to predators.

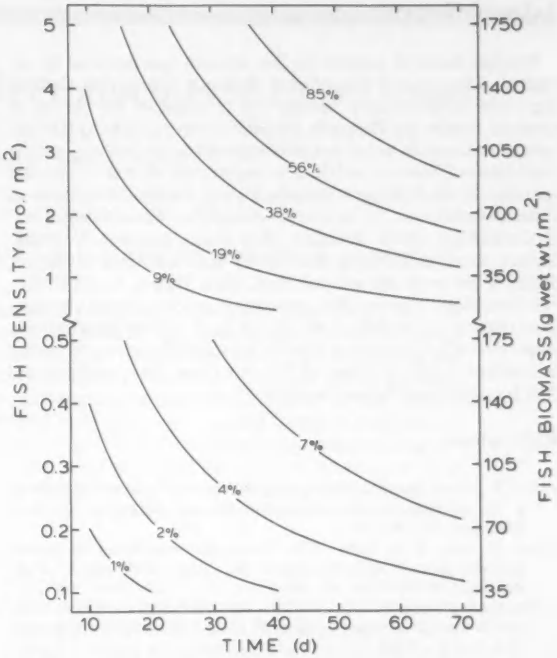

FIG. 9. Percent egg mortality as a function of both predator density and the time that demersal eggs are available as prey. The egg density used in this calculation was 4.7×10^6 eggs/m^2, which is the mean of the total egg deposition over the three field sessions (Table 1). $\bar{R}$ was assumed equivalent to 3.92% (dry body wt), $C = 0.5$, and dry body wt = 0.2 (wet body wt). Fish density and the equivalent biomass generally ranged between 0.1–0.5 fish/m^2 inshore and 1–5 fish/m^2 offshore at demersal spawning beds.

If we are correct in our assertion that published estimates of egg mortality due to predation at offshore demersal spawning sites are conservative, it follows that egg predation may be an important regulator of population size in species such as herring, sand lance (*Ammodytes* sp.), offshore spawning capelin, and other demersal spawners. In fact, given the demonstrated importance of egg and larval mortality on year-class formation, high levels of egg predation may be more important in regulating population size than predation on juveniles or adults, the net effect of such predation being a reduction in the reproductive capacity of the prey population.

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Zooplankton and Potential Fish Production in Lake Ontario

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Borgmann, U., H. Shear, and J. Moore. 1984. Zooplankton and potential fish production in Lake Ontario. Can. J. Fish. Aquat. Sci. 41: 1303–1309.

We calculated the annual production of epilimnetic crustacean zooplankton in Lake Ontario from data on the abundance of each species and its eggs, and from predictions of egg development times. *Bosmina longirostris* is the most productive species at the nearshore stations whereas *Diacyclops thomasi* is the most productive offshore. Total crustacean zooplankton production is fairly uniform throughout the lake, both nearshore and offshore, with the possible exception of somewhat reduced production at the western end of the lake. Average total production was estimated at $15\ g \cdot m^{-2} \cdot yr^{-1}$. Using a recent estimate of particle-size-conversion efficiency in Lake Ontario, obtained from data on contaminant concentrations in aquatic biota, we predicted potential fish production in the lake for various sizes of fish. From published data on the commercial fish harvest from 1910 to 1930, a time period when commercial production was at a maximum, we estimate that the past commercial harvest for pelagic species may have been 20% or more of our predicted potential production.

On a calculé la production annuelle des crustacés zooplanctoniques épilimnétiques du lac Ontario d'après les données sur l'abondance de chaque espèce et de ses œufs, et les prédictions du temps de développement des œufs. *Bosmina longirostris* et *Diacyclops thomasi* sont les espèces les plus productrices dans les zones côtières et hauturières respectivement. La production totale de crustacés zooplanctoniques est passablement uniforme dans tout le lac, que ce soit près du rivage ou au large, sauf peut-être dans la partie occidentale où la production est quelque peu réduite. On situe la production totale moyenne à $15\ g \cdot m^{-2} \cdot a^{-1}$. À partir d'une estimation récente de l'efficacité de conversion des particules selon leur taille, dans le lac Ontario, estimation générée par des données sur les concentrations de contaminants dans la flore et la faune aquatiques, on prédit la production potentielle de poissons selon différentes tailles. De plus, selon des données publiées sur la pêche commerciale effectuée de 1910 à 1930, période de production commerciale maximum, on établit que l'exploitation commerciale des espèces pélagiques s'élevait peut-être autrefois à 20 % ou plus de la production potentielle actuellement prévue.

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Data on zooplankton biomass and production in Lake Ontario have recently been collected by the surveillance program of the Great Lakes Fisheries Research Branch. The purpose of this project, and the purpose of related projects such as contaminant analyses in Great Lakes biota (Borgmann and Whittle 1983), is to provide information that will lead to a better understanding of trophic relationships in the lakes, possible effects of contaminants on these relationships, and the health of the lakes as a whole. In this paper we attempt to analyze the data on zooplankton production in Lake Ontario in a way that allows prediction of production at higher trophic levels.

To relate plankton production to real or potential fish production, we require a food web or food chain model that incorporates estimates of conversion efficiency (production/food consumption). The simplest food chain models assume a constant conversion efficiency from one trophic level to the next (e.g. 10%). However, applying such a model necessitates the identification of trophic interactions (who feeds on what). This may become difficult for natural ecosystems that are often composed of food webs rather than food chains. To circumvent this problem, Borgmann (1982) introduced the concept of particle-size-conversion efficiency (ϵ), defined as

$$(1) \quad \epsilon = \frac{\log (C_j/P_j)}{\log (W_j/W_i)}$$

where C_j is food consumed and P_j is biomass produced by a predator. Parameters W_j and W_i are the size of the predator and its prey, respectively. Parameter ϵ is a measure of the efficiency of conversion of biomass from one size to another, rather than from one trophic level to another. This is equivalent to the slope of the log of the biomass remaining (C_j at size W_i, and P_j at size W_j) plotted against the log of body size (e.g. Borgmann 1982, fig. 2). (Note that this is not equal to the regression line obtained by plotting $\log (C_j/P_j)$ against $\log (W_j/W_i)$ for different predator–prey species combinations unless the line is forced through the origin.) A review of the literature indicates that for carnivorous zooplankton, ϵ varies no more, and possibly less, than $\log (P/C)$ (Borgmann 1982). We suggest that it is just as valid to assume a constant ϵ as it is to assume a constant value for P/C when modelling pelagic ecosystems. This view is at least partially supported by the suggestion that fish conversion efficiency is related to prey size, with a higher efficiency (P/C)

resulting from a smaller predator–prey size ratio (e.g. Paloheimo and Dickie 1966; Kerr 1971).

Conversion efficiency is not a function of body size alone, and the assumption of a constant ϵ is therefore only an approximation. Furthermore, particle-size-conversion efficiency becomes meaningless for predator–prey size ratios near unity, because equation 1 predicts that conversion efficiency then becomes 100%. However, in pelagic ecosystems primary production occurs at microscopic sizes and production of large fish depends on a food web where most animals feed on organisms considerably smaller than themselves. Under these conditions, the assumption of a constant ϵ becomes a very powerful approximation because it greatly simpliflies the mathematics of calculating biomass flow up food webs (Borgmann 1982, 1983).

Particle-size-conversion efficiency in Lake Ontario has recently been estimated by Borgmann and Whittle (1983) using data on PCB and DDT concentrations for organisms ranging in size from zooplankton to coho salmon (*Oncorhynchus kisutch*) and lake trout (*Salvelinus namaycush*). The relationship between body size and the concentration of those contaminants that are accumulated primarily through the food chain is directly related to ϵ. This provides a simple method of estimating ϵ and describing contaminant concentrations in aquatic biota. In fact, it is easier to estimate ϵ than P/C from data on contaminant concentrations in natural systems because only the size and not the trophic level of a species needs to be known. This provides us with the estimate of ϵ (0.26, Borgmann and Whittle 1983) needed to predict production and biomass flow up the food web from zooplankton to fish in this lake.

Borgmann (1982) demonstrated that the flow of biomass up the food chain (F) can be described by the size corrected production (P_i*):

$$(2) \quad F = P_i^* = P_i W_i^{\epsilon}$$

This assumes that all production at one trophic level is consumed by the next, and therefore, size corrected production at one trophic level is equal to size corrected production at the next. This is because P_i is then equal to C_j in equation 1. Consequently, production at any trophic level can be calculated from rearrangement of equation 2:

$$(3) \quad P_i = F/W_i^{\epsilon}$$

We can use equations 2 and 3 to predict fish production from epilimnetic zooplankton production in Lake Ontario if we assume that the zooplankton species do not feed on one another and that all production at lower trophic levels passes through these species before being consumed by higher trophic levels. The first assumption is not entirely met, since cyclopoid copepods are carnivorous and will feed on small crustaceans (e.g. copepod nauplii). However, the small size range of the epilimnetic crustacean zooplankton (≈0.1–10 μg dry weight) suggests that carnivorous feeding by any of these species may be directed primarily towards rotifers and protozoans, rather than other crustaceans. The second assumption may lead to errors, if production by larger species such as *Mysis relicta* results from consumption of food smaller than crustacean zooplankton. Size corrected production of *M. relicta* in Lake Ontario has been estimated at $0.188\ g^{1.26} \cdot m^{-2} \cdot yr^{-1}$ (Borgmann 1983). Part of this may represent production that must be added to the zooplankton production to obtain an accurate estimate of F. However, because mysids feed on zooplankton (e.g. Bowers and Vanderploeg 1982), at least some of the mysid production is already included in the zooplankton size corrected production estimate. For simplicity, we will ignore production by mysids for now.

Zooplankton Production in Lake Ontario: Method of Calculation

Zooplankton were collected using a 30-cm diameter, 70-μm mesh net hauled vertically from the thermocline to the surface, or through the upper 20 m, whichever was less. Samples were collected weekly from station 12 (western end of Lake Ontario, 43°30′19″N, 79°21′19″W), station 93 near Niagara on the Lake (43°20′10″N, 78°52′10″W), station 41 at midlake (43°43′18″N, 78°01′01″W), and station 81 in the eastern basin (44°01′15″N, 76°40′27″W) from the end of March to the beginning of December in 1981. Samples were also collected every 2 or 3 wk at stations 22 (43°17′50″N, 79°00′15″W) and 40 (43°35′29″N, 78°00′42″W), which are near stations 93 and 41, respectively, to serve as "duplicates" for these stations (Fig. 1). Samples were preserved in 4% sugared formalin prior to identification and enumeration. Later, they were made up to a constant volume and mixed thoroughly before removing an aliquot with a 5-mL Hensen-Stemple pipet. This aliquot was transferred to a gridded counting dish and a portion of the dish area was counted. The amount of the original sample counted varied from the whole sample for rare species to as little as 0.0625% for the most abundant species (J. M. Cooley, Great Lakes Fisheries Research Branch, Burlington, Ont.).

To calculate production, the 3-point running average of the abundance of eggs and adult Cladocera was used. Copepod nauplii and copepodites, as well as a few loose eggs, which were not identified to species, were assigned to a species on the basis of the relative abundance of adult copepods. The 3-point running average of copepod eggs, nauplii, copepodites, and adults was then calculated as for the Cladocera.

The egg development time was estimated using the equation

$$K = (T - \alpha)^{\beta}/24$$

where K is the development time in days and T is the mean epilimnion temperature. Parameters A, α, and β are the Bělehrádek's coefficients relating development time to temperature. These were provided by J. M. Cooley (Cooley 1978; and pers. comm.) and are summarized in Table 1.

The instantaneous rate of population increase between two dates was calculated from

$$r = \ln (N_2/N_1)/t$$

where N_1 and N_2 are the number of adults plus immature individuals of a species at the beginning and end of the time interval (t). The instantaneous rate of reproduction (i.e. hatching) was calculated using the equation of Paloheimo (1974):

$$b = \frac{\ln (E + 1)}{K}$$

where b is the birth rate, K is egg development time, and E is the mean ratio of eggs to adults plus juveniles at the beginning and end of the time interval. The total number of animals hatched during a time interval was estimated from

$$B = \frac{b}{r}(N_1)(e^{rt} - 1) = \frac{b}{r}(N_2 - N_1) \qquad r \neq 0$$

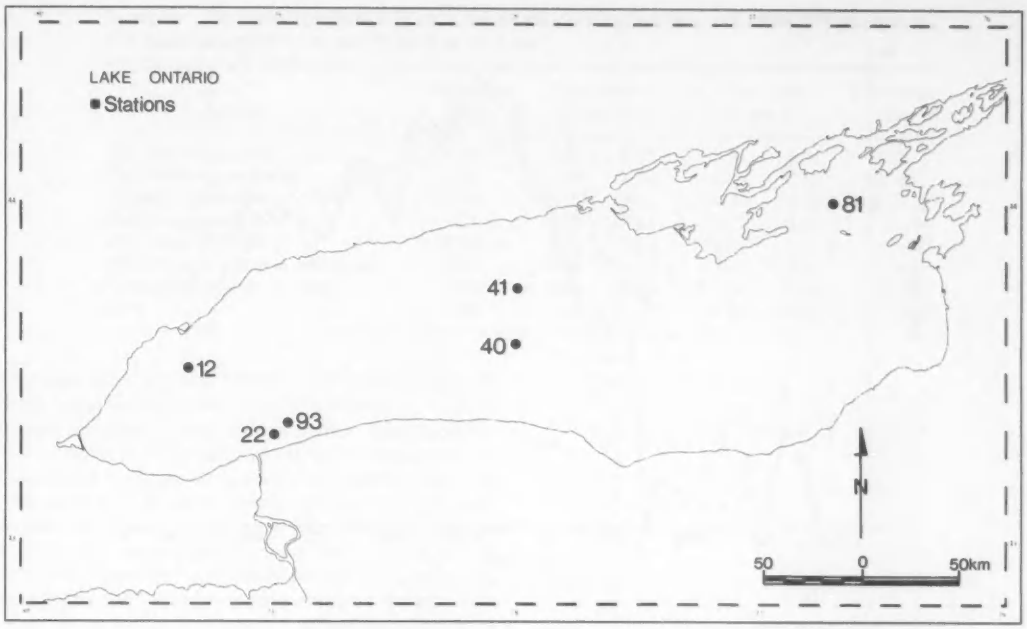

FIG. 1. Location of sampling stations for zooplankton in Lake Ontario.

TABLE 1. Mean dry weights for zooplankton and Bělehrádek coefficients for egg development times used in the production calculations.

Species	Estimated mean dry weight (μg)	Bělehrádek coefficients		
		A	α	β
Bosmina longirostris	1.2	3.751×10^6	−15.4	−3.11
Ceriodaphnia lacustris	1.9	1.763×10^3	3.7	−1.28
Daphnia retrocurva	4.2	6.591×10^4	−6.1	−2.12
Eubosmina coregoni	1.8	3.751×10^6	−15.4	−3.11
Diacyclops thomasi	3.2[a]	1.890×10^4	−4.8	−1.77
Tropocyclops prasinus mexicanus	1.7[a]	1.890×10^4	−4.8	−1.77
Calanoid copepods (pooled)	9.0[a]	3.847×10^4	−3.7	−2.00

[a]Adults only (see text).

where B is the number of new recruits and N_1 and N_2 are the number of adults plus juveniles at the beginning and end of the time interval. This is equivalent to Paloheimo's (1974) equation 3. Parameter B cannot be calculated this way if $r = 0$. If r is 0, $N_1 = N_2$, and B is given by

$$B = b(N)t \qquad r = 0$$

where t is the time interval. In practice we used this equation whenever r was less than 0.005 and set N equal to the mean of N_1 and N_2.

To estimate biomass production rates, we multiplied the total number of animals recruited during a time interval by the mean dry weight for individuals of each species during the same period. This was assumed constant for the Cladocera (see Table 1) and was calculated for copepods assuming a mean egg weight of 0.1 μg, naupliar weight of 0.2 μg, and copepodite weight of 1.9 μg. Copepod adult mean weights are listed in Table 1. These are the mean of unpublished data from our laboratory (J. B. Wilson and N. H. F. Watson, pers. comm.). For production purposes, overall mean individual copepod weight was calculated by dividing the total biomass (including eggs) by the number of adults plus juveniles (excluding eggs), thereby treating each egg bearing adult and its eggs as a single entity (because each adult plus its eggs are a single food item to a predator).

Production was estimated on a volume basis (grams per cubic metre) for each month and was then multiplied by the mean epilimnion depth at each station for each month to obtain areal production. Total annual production was calculated from the sum of the monthly areal production estimates. Size corrected production was calculated from the monthly production multiplied by $W^{0.26}$ where W is the monthly mean weight. This was also summed over the entire year.

In the above calculations we did not truncate the data to correct for unrealistic conditions. Since the instantaneous rate of population increase is $r = b - d$ (the difference between instantaneous recruitment and death rates) and since the death rate cannot be negative, b/r cannot have values between 0 and

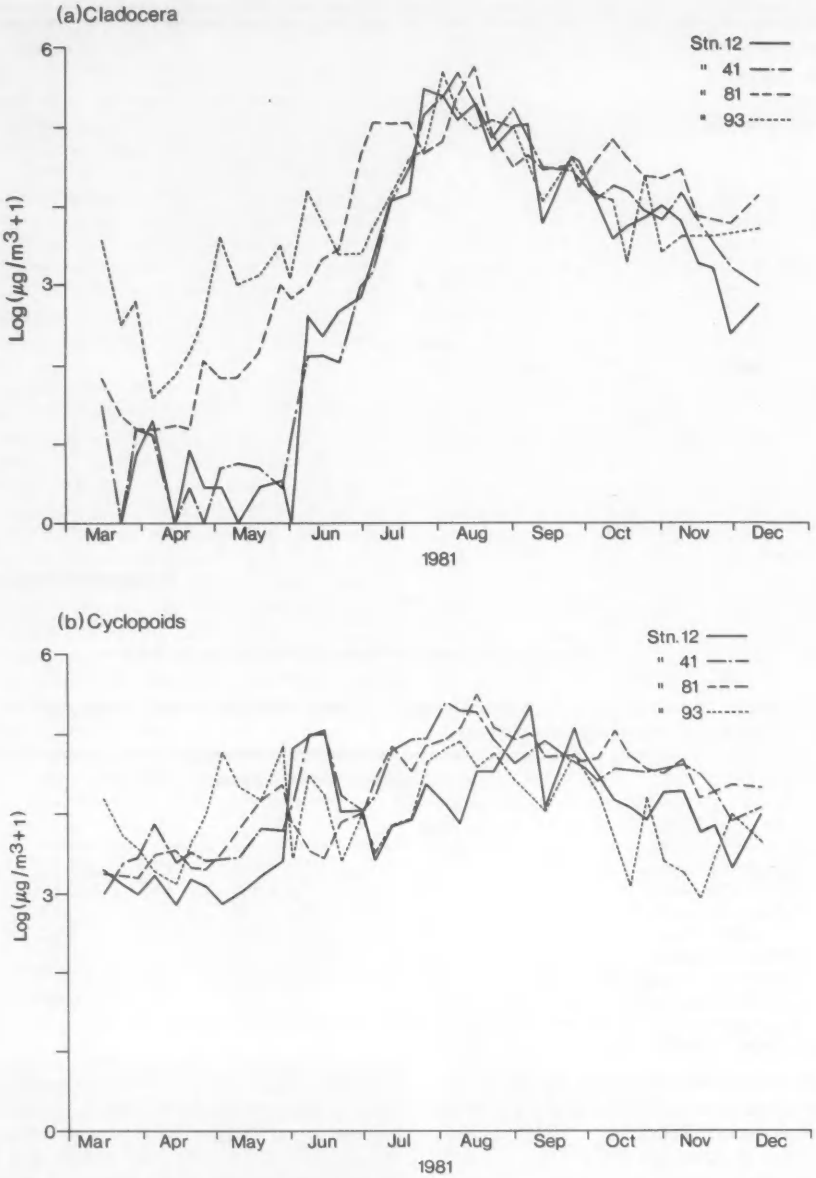

FIG. 2. Seasonal changes in biomass of (a) Cladocera and (b) cyclopoids at the four lake stations sampled weekly. These are single measurements, not running averages.

1. However, we did not set $b/r = 1$ (i.e. $d = 0$) whenever the calculated value of b/r was between 0 and 1 (i.e. $d < 0$). Instead, we assumed that this unrealistic condition was the result of sampling errors and that these would average out over the year. Truncating the data would overestimate production. As a check, we also calculated production using a crude method, assuming $\ln(E + 1) \approx E$. This is reasonable if E is much smaller than 1, and gives $B = b(N)t = (E/K)(N)t =$ (number of eggs)$(K^{-1})t$. This ignores data on adults and juveniles entirely and overestimates production slightly (e.g. by 23% if $E = 0.5$). Using this method the overall zooplankton production estimate was 25% greater, roughly as expected.

Results

Biomass of Cladocera and copepods at stations 12, 41, 81, and 93 at weekly intervals are shown in Fig. 2. These are unsmoothed data, before the 3-point running average was taken for production estimates. Cladoceran biomass varies much

TABLE 2. Total annual production ($g \cdot m^{-2}$, dry weight) for zooplankton at each station in Lake Ontario. The nearshore stations were near Niagara on the Lake.

Species	West end (12)	Near shore (22 and 93)		Center lake (40 and 41)		East basin (81)
Bosmina longirostris	1.91	6.92	6.29	3.21	4.01	3.63
Ceriodaphnia lacustris	0.62	1.98	1.54	0.96	0.69	0.96
Daphnia retrocurva	1.33	2.18	2.71	4.24	1.88	4.27
Eubosmina coregoni	0.21	0.10	0.11	0.22	0.31	1.43
Diacyclops thomasi	3.38	2.11	2.72	11.91	5.75	5.55
Tropocyclops prasinus mexicanus	0.47	1.40	1.32	0.49	0.26	1.49
Cananoid copepods (pooled)	0.06	0.64	0.64	0.06	0.04	0.11
Sum	7.98	15.33	15.33	21.09	12.94	17.44

more seasonally than copepod biomass. Temporal changes in biomass are fairly similar between the four stations.

Production estimates at each station for any species contributing 1% or more to the total production are summarized in Table 2. Calanoid copepods were treated as a group because of the low numbers of individuals in each species. Since production estimates are based on the egg ratio method, with no consideration of somatic growth rates and size at which mortality occurs, these are fairly crude estimates of production for those species that undergo a large increase in body size during their life cycle (i.e. copepods). Furthermore, the data in Table 2 are production estimates for the epilimnion only, and this will, therefore, not include production of cold-water zooplankton such as *Limnocalanus macrurus*.

Individual species of zooplankters appear to demonstrate different spatial patterns of production within the lake. These could be divided into four groups. *Bosmina longirostris*, *C. lacustris*, and the calanoid copepods, constituting the first group, have the highest production rates in the nearshore region (stations 22 and 93). *Bosmina longirostris* is responsible for the bulk of the production at the nearshore stations. By contrast, the other cladocerans, *D. retrocurva* and *E. coregoni*, which make up the second group, have low or moderate production at the nearshore station and maximum production in the east basin. This is particularly noticeable for *E. coregoni*. A third group, containing *T. prasinus mexicanus*, also has highest production in the eastern basin, but also has high production at the nearshore stations. Finally, *D. thomasi* (also called *Diacyclops biscuspidatus thomasi* — see Robertson and Gannon 1981), differs from these species in demonstrating highest production in the central part of the lake, but also has high production in the eastern and western basins of the lake. In fact, this copepod accounts for more production than any other species at all stations except the near shore. In spite of these substantial differences in production of individual species from station to station, the total production at any one station appears to be relatively constant, except for the western end of the lake. The prevailing winds in this region are from the west, which causes upwelling and lower mean summer temperatures in the western end of Lake Ontario. This might account for the lower production here.

The seasonal variation in production is shown in Fig. 3 for the three species with the highest production. *Bosmina longirostris* has a very pronounced peak in production in August. *Daphnia retrocurva* production is highest in August and September, slightly later than *B. longirostris*. *Diacyclops thomasi* production is highest in August but production is high from July to October inclusive, a longer period than for the Cladocera.

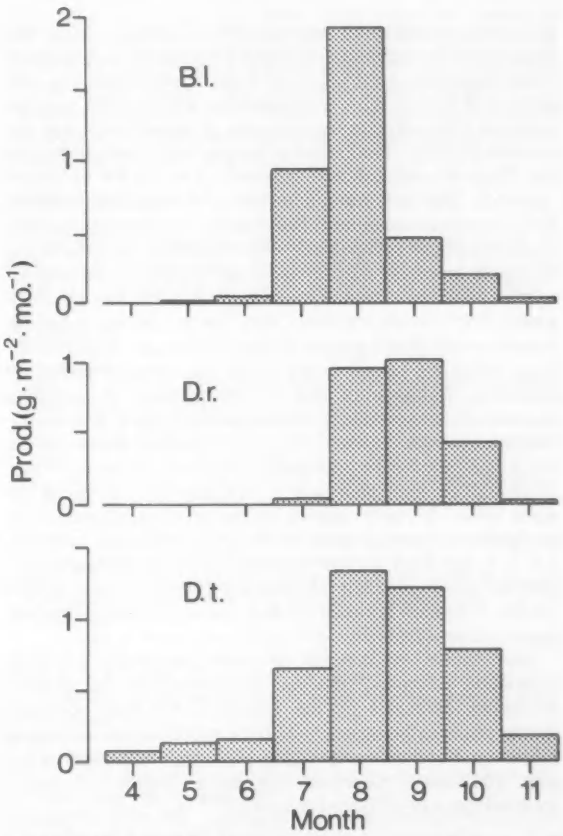

FIG. 3. Mean monthly biomass production (dry weight) for the three most productive zooplankton species in 1981. B.l., *Bosmina longirostris*; D.r., *Daphnia retrocurva*; D.t., *Diacyclops thomasi*.

Although production by *B. longirostris* in August is substantially greater than production by *D. thomasi* in the same month, the longer period of production for the copepod results in a greater annual production. The bulk of the annual production is accounted for during the sampling period (8 mo) (Fig. 3).

The mean annual production and mean biomass (for the sampling period) averaged over the six stations are shown in Table 3. Since the sampling stations were chosen to represent the major regions within the lake (i.e. nearshore, offshore or center lake, east basin, and west basin) and since total

TABLE 3. Annual production, mean biomass, the production to biomass ratio, and size corrected production (production·$W^{0.26}$) averaged over the six stations in Lake Ontario.

Species	Production ($g \cdot m^{-2} \cdot yr^{-1}$)	Biomass ($g \cdot m^{-2}$)	P/B (yr^{-1})	Size corrected production ($g^{1.26} \cdot m^{-2} \cdot yr^{-1}$)
Bosmina longirostris	4.33	0.154	28	0.125
Ceriodaphnia lacustris	1.12	0.020	56	0.037
Daphnia retrocurva	2.77	0.124	22	0.111
Eubosmina coregoni	0.40	0.016	25	0.013
Diacyclops thomasi	5.24	0.342	15	0.165
Tropocyclops prasinus mexicanus	0.90	0.166	5.4	0.027
Calanoid copepods (pooled)	0.26	0.008	32	0.010
Sum	15.02	0.830		0.488

production is similar for these stations (SD/mean = 29%), this should provide reasonable estimates for whole lake production. Total production was 15.02 $g \cdot m^{-2} \cdot yr^{-1}$ (our preliminary estimate of 18 $g \cdot m^{-2} \cdot yr^{-1}$, Borgmann and Whittle 1983, was too high due to an error in the computer program which was not detected initially). Production to biomass ratios were higher for the Cladocera and calanoid copepods than for the cyclopoid copepods. The very low production to biomass ratio obtained for *T. prasinus mexicanus* is likely due to an overestimate of the biomass because copepodites, which constitute the bulk of the cyclopoid biomass, were assigned to species on the basis of relative adult abundance. Errors incurred in this way are much greater for biomass estimates than for production estimates because production is primarily dependent on the abundance of eggs, which are carried by the adults and therefore positively identified. Inclusion of part of the apparent *T. prasinus mexicanus* biomass with *D. thomasi* would make P/B ratios for both species equal to about $12 \cdot yr^{-1}$. Part of the reason for the lower P/B ratio for the cyclopoids, relative to Cladocera, is that cyclopoids are more abundant in the winter months, raising the mean annual biomass relative to that of the Cladocera. The production to biomass ratios for the month of August were 3.9, 3.8, 2.7, and 3.4 $\cdot mo^{-1}$ for *B. longirostris*, *D. retrocurva*, *D. thomasi*, and *T. prasinus mexicanus*, respectively, indicating a smaller difference between Cladocera and cyclopoids than the annual values (Table 3).

Also shown in Table 3 are the annual size corrected production estimates, obtained by multiplying the monthly production estimates by the average dry weight for each species raised to the exponent 0.26 (the particle-size-conversion efficiency, Borgmann and Whittle 1983) and summing over the year. The total size corrected production for the epilimnetic zooplankton was estimated at 0.488 $g^{1.26} \cdot m^{-2} \cdot yr^{-1}$.

Potential Fish Production in Lake Ontario

Our estimate of F, using equation 2, is 0.488 $g^{1.26} \cdot m^{-2} \cdot yr^{-1}$ (Table 3). Lake Ontario has an area of about 19.5×10^9 m^2, which gives us an estimate for total size corrected production of about 9.5×10^9 $g^{1.26} \cdot yr^{-1}$. Using this estimate of F, and equation 3, potential fish production for various sizes of fish can be calculated. Some examples are listed in Table 4. For example, the lake could produce 1.9×10^9 $g \cdot yr^{-1}$ of 500 g dry weight (approx. 2 kg wet weight) fish, or slightly more if fish are harvested at half that size. Commercial production for smaller fish (5 g dry weight) could be 6.2×10^9 $g \cdot yr^{-1}$ (Table 4). If fish are harvested at several sizes, then some of the production of the smaller fish must be left to feed the larger fish. In this case, the sum of the total commercial production for each size-class multiplied by the fish size raised to the exponent 0.26 must equal 9.5×10^9 $g^{1.26} \cdot yr^{-1}$, as shown in Table 4. These calculations refer to potential production and assume that nonpredation mortality of zooplankton and higher trophic levels is negligible. As data on nonpredation mortality becomes available in the future (e.g. alewives, *Alosa pseudoharengus*, washing up on the beaches in spring), the true production can be calculated by substracting nonpredation mortality, on a size corrected basis, from F, in a way analogous to that used to calculate potential production for more than one size-class.

TABLE 4. Potential annual fish production in Lake Ontario assuming that biomass flow up the food chain is 9.5×10^9 $g^{1.26} \cdot yr^{-1}$.

Fish size W (g dry weight)	Potential production $P = 9.5 \times 10^9 / W^{0.26}$ (g dry weight)[a]	No. of fish P/W
500	1.9×10^9	3.8×10^6
250	2.3×10^9	9.2×10^6
5	6.2×10^9	1240×10^6
5 plus 500	(3 plus 0.98) $\times 10^9$	(600 plus 1.96) $\times 10^6$

[a]The last value in this column was calculated as follows: 3×10^9 g $\times$ $5^{0.26}$ $g^{0.26} = 4.56 \times 10^9$ $g^{1.26}$ size corrected production for 5-g fish; $(9.5 - 4.56) \times 10^9 = 4.94 \times 10^9$ $g^{1.26}$ remaining; 4.94×10^9 $g^{1.26}$/ $500^{0.26} = 0.98 \times 10^9$ g production for 500-g fish.

Our estimates of potential fish production can be compared with actual fish harvests in the past. (There is little commercial fishing at present.) Greatest harvests in the past occurred from about 1910 to 1930 and consisted primarily of lake trout, herring (*Coregonus artedi*) and ciscoes (*Coregonus* sp.), and lake whitefish (*Coregonus clupeaformis*) (Christie 1972, fig. 2 and 3). Recorded commercial harvests of other species are minimal, except in the Bay of Quinte. We calculated average annual harvests for these fish from the appendix tables provided by Christie (1973), excluding the Bay of Quinte because this is a small, shallow bay where fish production does not necessarily result primarily from production through a pelagic food chain. We do not know what size these fish were, but estimated mean sizes of 200 g (wet weight) for herring and ciscoes and 2 kg for lake trout and lake whitefish, based in part on mean sizes of fish given by Scott and Crossman (1973). This gives us an estimate of past commercial production (from harvests) of about 0.261×10^9 $g \cdot yr^{-1}$ (dry weight) or 1.145×10^9 $g^{1.26} \cdot yr^{-1}$ (Table 5). This is about 12% of our estimated potential production using zooplankton data from 1981. The commercial fish harvest

TABLE 5. Average annual commercial fish production in Lake Ontario, excluding the Bay of Quinte, from 1910 to 1930. Calculations on a dry weight basis assuming 75% moisture content.

Species	Production (10^9 g·yr^{-1})	Estimated mean size (g)	Size corrected production (10^9 g$^{1.26}$·yr^{-1})
Lake trout	0.0749	500	0.377
Lake herring and ciscoes	0.0746	50	0.206
Lake whitefish	0.1117	500	0.562
Sum	0.2612	—	1.145

[a]Calculated from data of Christie (1973).

estimates, however, are low because not all fish caught were recorded. Burbot (*Lota lota*) were apparently abundant in the 1920s and U.S. fishermen reported annual catches of 60 000 lb (27 000 kg) as compared with only 45 000 lb (20 000 kg) for lake trout (Christie 1973). The real commercial harvest might therefore have been closer to 2×10^9 g$^{1.26}$·yr^{-1}. This suggests that the total commercial harvest may have been as high as 20% or more of our estimated potential production. This indicates that our estimates of potential fish production may be fairly accurate, bearing in mind that potential production includes natural mortality. If the fishery can be managed carefully in such a way that natural mortality at intermediate trophic levels is reduced (i.e. the food resources are fully utilized), then some increase in commercial fish production above past levels (i.e. Table 5) may be possible.

Another application of our calculations is the estimation of ideal stocking rates for the lake. Much of the large fish production in Lake Ontario is due to stocked salmonids (e.g. coho and chinook salmon (*Oncorhynchus tshawytscha*), lake trout, etc.), some of which cannot reproduce within the lake. The calculations in Table 4 present production for various sizes of fish and, hence, total numbers of fish that could be harvested from the lake annually. For 250- to 500-g (dry weight) fish, total potential production would be roughly 4×10^9 to 9×10^6 fish (Table 4). The total number of salmonids stocked by Ontario and New York State in 1981 was 4.5×10^6 fish (Lake Ontario Salmonid Stocking Summaries, OMNR). This is already roughly equal to the maximum number of fish that can be harvested annually. Calculation of the exact number of fish that should be stocked must include, of course, some estimate of mortality of the stocked fish before they reach harvestable size. It must also take into consideration that potential fish production is calculated on the basis of complete utilization of the food resource, a condition that may be approached, but probably rarely actually reached.

Discussion

We have estimated potential fish production from zooplankton production using simple equations requiring no direct estimate of the number of trophic levels between these organisms. Our main assumptions are that particle-size-conversion efficiency is relatively constant at 0.26 and that nonpredation mortality is negligible from zooplankton to fish in the Lake Ontario ecosystem. Because of the latter assumption, we refer to our estimates of fish production as potential fish production.

Sheldon et al. (1977) compared fish production and primary production in marine ecosystems using a model containing concepts similar to what we have discussed here. Their calculations were based on the assumption that the biomass size spectrum was fairly flat. They showed that this is a reasonable assumption if predator–prey size ratios and growth efficiencies are relatively constant, if there is an allometric relationship between body size and growth rate, and if most of the production at one trophic level is consumed by the next. Other authors have expanded the theoretical analysis of the biomass size spectrum and tried to show why it should have a well-defined shape (e.g. Kerr 1974; Platt and Denman 1978; Silvert and Platt 1980). However, less data have been collected on the shape of the biomass size spectrum in freshwaters, especially over large size ranges (i.e. many trophic levels). Our calculations bypass the need for specifying the shape of the biomass size spectrum in Lake Ontario (about which we have little data) and require no prediction for the production/biomass ratios at the different trophic levels. Furthermore, while we have no data on predator–prey size ratios or conversion efficiencies independently in the lake, we do have an estimate of particle-size-conversion efficiency from data on contaminant concentrations in the biota (Borgmann and Whittle 1983). Particle-size-conversion efficiency is therefore a convenient concept for estimating potential production of fish through the pelagic food web of Lake Ontario.

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Influence of Temperature and Light on Rates of Inorganic Nitrogen Transport by Algae in an Arctic Lake[1]

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Whalen, S. C., and V. Alexander. 1984. Influence of temperature and light on rates of inorganic nitrogen transport by algae in an arctic lake. Can. J. Fish. Aquat. Sci. 41: 1310–1318.

Nitrogen-15 tracer techniques were used to assess the individual effects of temperature and light on maximum rates of dissolved inorganic nitrogen (DIN) transport by phytoplankton in an arctic lake. Transport rates of NO_3^- and NH_4^+ as a function of temperature in the range 3–30°C were adequately described by second- or third-order polynomial functions. The optimum temperature for DIN transport was greater than the ambient water temperature by as much as 13°; thus, transport at the ambient water temperature averaged only 55–60% of optimum. Mean temperature coefficients ($\bar{X} \pm$ SD) for NO_3^- and NH_4^+ transport in the temperature range >0–15°C were 2.3 ± 0.5 and 2.4 ± 0.3. The ratio of dark to light-saturated DIN transport (ρ_D/ρ_L) was used as a measure of light dependence in maximum rates of DIN transport. Values of ρ_D/ρ_L ($\bar{X} \pm$ SD) for NO_3^- and NH_4^+ were 0.15 ± 0.09 and 0.41 ± 0.11, indicating a stronger light dependence for NO_3^- than NH_4^+ transport. In cases where dark NO_3^- transport was negligible, light was treated as a substrate and the Michaelis–Menten relationship fitted to the data. The half-saturation constant for light intensity in maximum NO_3^- transport ranged from 7 to 29 $\mu E \cdot m^{-2} \cdot s^{-1}$, which was 6–31% of the photosynthetically active radiation at the lake surface.

On a utilisé des techniques de traçage à l'azote 15 pour évaluer l'incidence individuelle de la température et de la lumière sur les taux maximum de transport de l'azote inorganique dissous (AID) par le phytoplankton d'un lac de l'Arctique. Des équations polynomiales du 2e et du 3e degrés décrivaient adéquatement les taux de transport du NO_3^- et du NH_4^+ en tant que fonction de la température variant de 3 à 30°C. La température optimum pour le transport de l'AID peut s'élever jusqu'à 13° au-dessus de la température ambiante de l'eau. Par conséquent, le transport à la température ambiante de l'eau n'atteignait en moyenne que 55 à 60 % de l'optimum. Les coefficients de la température moyenne ($\bar{X} \pm$ É.-T.) pour le transport du NO_3^- et du NH_4^+, quand la température variait de 0 à 15°C, s'élevaient à 2,3 ± 0,5 et à 2,4 ± 0,3 respectivement. Le rapport entre le transport nocturne et le transport photosaturé de l'AID (ρ_N/ρ_P) a été utilisé comme mesure de la dépendance lumineuse des taux maximum de transport de l'AID. Les valeurs de ρ_N/ρ_P) ($\bar{X} \pm$ É.-T.) pour NO_3^- et NH_4^+ équivalaient à 0,15 ± 0,09 et à 0,41 ± 0,11 respectivement, ce qui porte à croire à une plus forte dépendance lumineuse du transport du NO_3^- que celui du NH_4^+. Dans les cas où le transport nocturne du NO_3^- était négligeable, la lumière a été considérée comme un substrat et la relation Michaelis–Menten a été ajustée selon les données. La constante de semi-saturation pour l'intensité lumineuse au cours du transport maximum du NO_3^- variait de 7 à 29 $\mu E \cdot m^{-2} \cdot s^{-1}$, ce qui représente de 6 à 31 % de la radiation photosynthétiquement active à la surface du lac.

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Nitrogen uptake dynamics in natural populations of marine phytoplankton has been extensively studied using ^{15}N-labelled substrates (cf. reviews by Collos and Slawyk 1980; McCarthy 1980). However, this aspect of algal ecology has been largely unexplored in lacustrine environments. The few reported investigations using ^{15}N tracers in lakes and reservoirs to quantify rates of dissolved inorganic nitrogen (DIN = NO_3^- + NH_4^+) uptake by phytoplankton have focused primarily on seasonal patterns of uptake and nitrogenous nutrient preferences of the populations studied (Toetz et al. 1977; Liao and Lean 1978; Murphy 1980; Axler et al. 1982; McCarthy et al. 1982). Only the studies of Procházková et al. (1970) and Cole and Toetz (1979) have addressed in any detail the influence of light on DIN uptake in freshwater plankton communities. Furthermore, field studies concerned with the effects of temperature on algal DIN uptake in lakes are lacking.

The present investigation was conducted at Toolik Lake in the Alaskan arctic during the summers of 1980–82. Our goals were to (1) describe quantitatively changes in maximum or nitrogen-saturated (i.e. NO_3^- or NH_4^+ in excess when measuring NO_3^- or NH_4^+ uptake, respectively) rates of DIN uptake by algae relative to changes in water temperature, (2) relate seasonal changes in the optimum temperature for N-saturated uptake of DIN to ambient water temperatures, (3) test for a light dependence in maximum rates of DIN uptake, (4) calculate half-saturation constants for light intensity in maximum DIN uptake when a light dependence was evident, and (5) use results of our study to predict the influences of light and temperature on DIN uptake at ambient nutrient levels of the lake.

[1]Institute of Marine Science Contribution No. 548.

Materials and Methods

Toolik Lake (68°38′N; 149°38′W) is a large (1.5 km^2), deep ($\bar{z}$ = 7 m) arctic lake located along the Trans-Alaska Pipeline corridor in the north-facing foothills of the Brooks Range. The lake is very unproductive (mean chlorophyll a = 1.3 $\mu g \cdot L^{-1}$; ^{14}C primary production = 8 g $C \cdot m^{-2}$, June through August) and nutrient poor. During the ice-free period (late June through September), concentrations of nitrate, ammonium, and total dissolved phosphorus are about 0.10, 0.15, and 0.15 $\mu mol \cdot L^{-1}$ throughout the water column, while levels of soluble reactive phosphorus are generally undetectable (<0.03 $\mu mol \cdot L^{-1}$). Thermal stratification is evident for about a 5-wk period in July and August and the surface water temperature reaches about 15°C. Nitrogen plays an important role in the phytoplankton ecology of Toolik Lake. Our kinetic experiments (Whalen and Alexander 1984) indicate that uptake is limited by the external DIN concentration and (unpublished) results of 24-h bioassay experiments show that N-enrichment stimulates photosynthesis as often as P-enrichment.

All water was collected from a depth of 3 m (approximately 10–15% surface light penetration) in the central lake basin with a submersible pump and filtered through 202-μm Nitex mesh prior to experimental manipulation. Portions of Nitex-filtered water were reserved for duplicate chlorophyll a (fluorometric technique; Strickland and Parsons 1972) and particulate nitrogen (PN) (modified Solórzano and Sharp 1980) assays. Determinations of ambient $NO_3^- + NO_2^-$ (hereafter referred to as NO_3^-) and NH_4^+ were made by Cu–Cd reduction and the phenolhypochlorite methods, respectively, following the Auto-Analyzer techniques of Whitledge et al. (1981). The method of sample collection had no significant effect (Student's t-test; df = 18, $P > 0.05$, all cases) on biological activity in terms of $H^{14}CO_3^-$, $^{15}NO_3^-$, or $^{15}NH_4^+$ uptake when periodically compared with replicate Van Dorn-collected samples from the same depth.

In experiments to assess temperature dependence in DIN uptake, 30 samples were collected at midnight into 1.3-L clear polystyrene bottles. A series of aquarium heaters and cryocool units served to maintain five approximately 1-m^3 outdoor, water-filled incubators at constant temperatures ranging from 3 to 29°C, with one incubator always held at the 2-m lake temperature by continual exchange with water from that depth. Over an 8-h acclimation period, sample bottles were gradually stepped up or down through the temperature range of the incubators such that each incubator finally held six bottles. Experiments were then begun by adding 3.07 $\mu mol \cdot L^{-1}$ of 99 atom-% enriched $Na^{15}NO_3$ to half the samples in each incubator, the other half receiving a similar quantity of 99 atom-% enriched $^{15}NH_4Cl$. Incubators were screened such that 50% of the incident irradiance reached the sample bottles.

Experiments to measure light dependence in DIN uptake were begun within 1 h of sampling (08:00, local time) by adding 3.33 $\mu mol \cdot L^{-1}$ of 99 atom-% enriched $Na^{15}NO_3$ or $^{15}NH_4Cl$ to 2.4-L clear and opaque polystyrene bottles, as well as to similar sized bottles covered with neutral density screens (Perforated Products Inc. No. 15G, 10/40, 40P, 15S, 40T, and 50W allowing 48, 25, 12, 9, 7, and 1% light transmission, respectively). For each DIN species, duplicate bottles of each simulated light level were suspended horizontally in the lake at 0.25 m. Simultaneously, photosynthetically active radiation (PAR) at 0.25 m was measured with a Lambda underwater quantum sensor (Li 192S) and recorded with a digital integrator (Li-500).

Experiments were terminated after 8 h (temperature) or 24 h (light) by low vacuum filtration (<20 cmHg) onto precombusted (6 h at 450°C) Gelman A/E glass fiber filters. Filters were rinsed with 25–50 mL of filtered lake water, frozen, and later dried at 60°C. Samples were converted to N_2 by a modified Dumas method and assayed for ^{15}N incorporation in a Bendix Time-of-Flight model 17-210 mass spectrometer. Seston enrichments ranged from 0.1 to 7.0 atom-% excess ^{15}N. Following Conway and Whitledge (1979), we have expressed our results as ρ, the (biological) transport rate, which is calculated by multiplying the nutrient-specific uptake rate, V (per hour), times the PN concentration (nanomoles per litre) at the start of the experiment. The units of ρ are therefore nanomoles nitrogen per litre per hour.

We stress that values of ρ reported here represent the maximum (nitrogen-saturated) response in DIN transport with respect to the manipulated variable, PAR, or temperature. That is, based upon results of our kinetic experiments (Whalen and Alexander 1984), DIN was in adequate supply due to the addition of >3 $\mu mol \cdot L^{-1}$ of ^{15}N isotope (=20–30 times ambient $^{14}NO_3^-$ or $^{14}NH_4^+$). Thus, multiplicative or additive effects of DIN and light or temperature limitation were eliminated.

Results

Temperature Dependence Experiments

In all temperature dependence experiments, DIN transport (ρ_T) increased as temperature increased to an optimum, then declined markedly as temperature became supraoptimal (Fig. 1). A second- or third-order polynomial was fitted to the entire range of temperature-transport data for each experiment such that the addition of a higher term did not significantly (F test; $P > 0.05$) improve the model (Table 1). Optimum temperatures (T_{opt}) for DIN transport were calculated from these regression equations. For NO_3^- and NH_4^+, T_{opt} ranged from 16 to 22°C and from 19 to 23°C with respective mean values of 19 and 21°C (Table 1). The difference between the means was not significant (Student's t-test; df = 6, P = 0.22). The ambient water temperature, T_{amb}, ranged from 8 to 14°C with a mean of 11°C. Thus, the calculated values of T_{opt} for NO_3^- and NH_4^+ transport averaged 8 and 10°C higher than the measured ambient water temperature. Differences between the mean ambient water temperature and the mean optimum temperatures for NO_3^- and NH_4^+ transport were significant (Student's t-test; df = 6, $P < 0.01$, both cases). When the entire data were pooled, no significant correlation could be found between increasing values of T_{opt} and increasing values of T_{amb} (Spearman's rank correlation coefficient; r_s = 0.27, df = 6, P = 0.52). Because T_{opt} was approximately 9° higher than T_{amb}, DIN transport rates at T_{amb} were temperature limited (compare $\rho_{T(amb)}/\rho_{T(opt)}$ = transport efficiency, Table 1). Transport efficiency ranged from 0.45 to 0.83 and from 0.27 to 0.76 for NO_3^- and NH_4^+, with means of 0.61 and 0.56. The difference between means was not significant (Student's t-test; df = 6, P = 0.73).

The rate of NH_4^+ transport far exceeded that of NO_3^- at a given temperature in every experiment (Fig. 1). Values of $\rho_T(NH_4^+)/\rho_T(NO_3^-)$ ranged from 1.8 to 5.8 but remained generally constant at approximately 2–3 in all experiments until

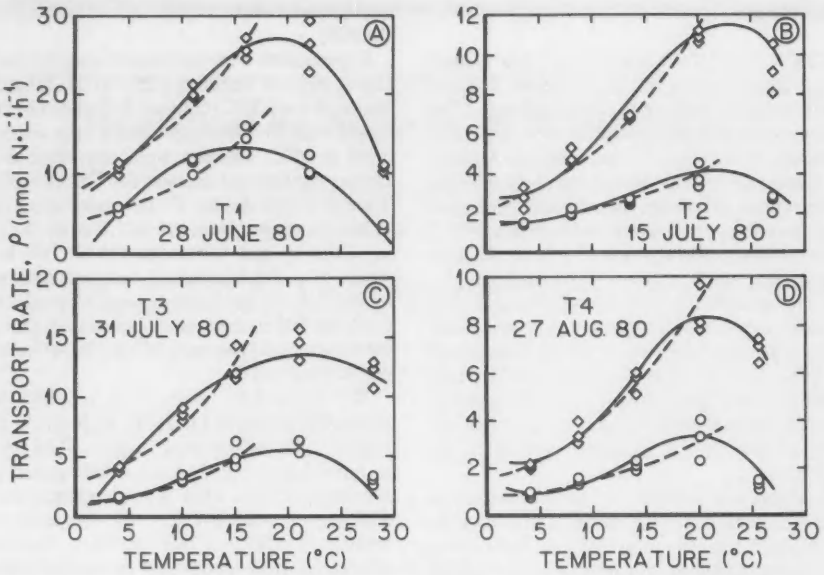

FIG. 1. Effect of water temperature on nitrogen-saturated rates of transport for NO_3^- (○) and NH_4^+ (◇), Toolik Lake phytoplankton. Broken curves are least-squares fits of exponential functions to data over the range of positive response in DIN transport with increasing temperature. Solid curves are least-squares fits of polynomials to entire data.

temperature became supraoptimal (Fig. 2). At that point, relative transport rates of NO_3^- declined more dramatically than those of NH_4^+ (Fig. 1), leading to the sharp increase in $\rho_T(NH_4^+)/\rho_T(NO_3^-)$ observed at approximately 25–29°C (Fig. 2). The anabolic pathway for NO_3^- is apparently more heat labile than its NH_4^+ counterpart.

An exponential model was fitted to the temperature-transport data over the range of positive response with increasing temperature for each experiment (Fig. 1), and Q_{10} values for DIN transport were calculated from the van't Hoff equation (Swan 1974). The temperature coefficients for NO_3^- transport ranged from 1.8 to 3.1, while those for NH_4^+ ranged from 2.1 to 2.8 (Table 1). Means ($\bar{X} \pm$ SD) were 2.3 ± 0.5 and 2.4 ± 0.3 for NO_3^- and NH_4^+, respectively. The difference between sample means was not significant (Student's t-test; df = 6, $P = 0.83$).

Light Dependence Experiments

The ratio of dark to light-saturated transport (ρ_D/ρ_L)was more variable for NO_3^- than NH_4^+ (Table 2). The ratio ρ_D/ρ_L ranged over a factor of 6 (0.005–0.31) for nitrate compared with a factor of 2 (0.27–0.57) for ammonium. The respective mean values ($\bar{X} \pm$ SD) of ρ_D/ρ_L for NO_3^- and NH_4^+ were 0.15 ± 0.09 and 0.41 ± 0.11 with the difference significant at $P < 0.01$ (Student's t-test; df = 16). Thus, the average ρ_D/ρ_L for ammonium was about 2.7 times that for nitrate. Substantial rates of dark transport were observed in experiments LT2 and LT6-8 for NO_3^- and in all cases for NH_4^+ (Table 2).

Typical response curves for NO_3^- and NH_4^+ transport as a function of PAR (Fig. 3) show that at all levels of irradiance rates of NH_4^+ transport were always greater than corresponding rates of NO_3^- transport. When transport data at all levels of PAR were expressed as percent of the maximum observed for each DIN species in a particular experiment (cases of obviously depressed transport in clear bottles omitted; see below), transport rates for NH_4^+ were still nearly always greater than for NO_3^- (data not shown). On occasions the data showed considerable scatter (Fig. 3A; NO_3^-), which caused difficulty in interpretation. It at first seems reasonable in many cases (e.g. Fig. 3B) to subtract values of DIN transport in the dark from those in the light and to fit a rectangular hyperbola to the data (MacIsaac and Dugdale 1972):

$$(1) \quad \rho = \rho_D + \rho_{max} \frac{I}{K_{LT} + I}$$

where ρ is the nitrogen-saturated rate of N transport measured at a PAR level of I, ρ_D is the nitrogen-saturated rate of N transport in the dark, ρ_{max} is the rate of N transport under conditions of nitrogen and light saturation, and K_{LT} is the value of I for which ρ is half of ρ_{max} (half-saturation constant). However, it has been suggested (MacIsaac and Dougdale 1972; MacIsaac et al. 1974) that dark DIN transport is not necessarily a constant proportion of light transport, but rather a distinct process that occurs in the dark or under low light conditions. MacIsaac and Dugdale (1972) chose to ignore the transport of DIN in the dark and fitted the Michaelis-Menten relationship to their light-nitrogen transport data as follows:

$$(2) \quad \rho = \rho_{max} \frac{I}{K_{LT} + I}$$

where all parameters are as previously defined. Nelson and Conway (1979) argued that equation (2) appropriately describes light-nitrogen transport data only when the data appear to conform to Michaelis–Menten kinetics *and* dark transport is a negligible proportion (<10%) of light-saturated transport.

Based on the criteria of Nelson and Conway (1979), equation (2) was suitable for describing the NO_3^- transport data from

TABLE 1. Regression analyses performed on data from Toolik Lake experiments
measured values of T_{amb}, DIN transport rates at T_{amb} ($\rho_{T(amb)}$) as well as calculated
of all regression equations are statistically significant at $P < 0.05$.

Experiment	Date	Regression analyses Equation
T1	28 June 1980	$\rho_T(NO_3^-) = 3.98(1.087)^T$
		$\rho_T(NO_3^-) = -1.440 + 1.882T - 0.060T^2$
		$\rho_T(NH_4^+) = 8.23(1.077)^T$
		$\rho_T(NH_4^+) = 7.285 + 0.481T + 0.110T^2 - 0.$
T2	15 July 1980	$\rho_T(NO_3^-) = 1.29(1.061)^T$
		$\rho_T(NO_3^-) = 1.831 - 0.210T + 0.034T^2 - 0.$
		$\rho_T(NH_4^+) = 2.26(1.085)^T$
		$\rho_T(NH_4^+) = 3.440 - 0.399T + 0.081T^2 - 0.$
T3	31 July 1980	$\rho_T(NO_3^-) = 0.92(1.120)^T$
		$\rho_T(NO_3^-) = 1.566 - 0.230T + 0.054T^2 - 0.$
		$\rho_T(NH_4^+) = 2.82(1.107)^T$
		$\rho_T(NH_4^+) = -1.241 + 1.345T - 0.031T^2$
T4	27 Aug. 1980	$\rho_T(NO_3^-) = 0.68(1.081)^T$
		$\rho_T(NO_3^-) = 1.953 - 0.453T + 0.056T^2 - 0.$
		$\rho_T(NH_4^+) = 1.54(1.093)^T$
		$\rho_T(NH_4^+) = 3.482 - 0.676T + 0.096T^2 - 0.$

[a]Calculated from the van't Hoff expression and corresponding least-squares fit of
[b]Temperature to nearest 1°C of greatest ρ predicted by corresponding least-square
[c]Measured water temperature to nearest 1°C in incubator allowing continuous exc
[d]Maximum value of ρ predicted from corresponding least-squares fit of polynom
[e]Measured mean value of ρ in incubator allowing continuous exchange of water

ments examining the temperature dependence of nitrogen-saturated DIN transport, ρ (nmol $N \cdot L^{-1} \cdot h^{-1}$). Also, culated values of Q_{10} and T_{opt}, DIN transport at T_{opt} ($\rho_{T(opt)}$), and transport efficiencies ($\rho_{T(amb)}/\rho_{T(opt)}$). Slopes

es		Temperature coefficient	Optimum temperature	Ambient temperature	Transport rates		
	r^2	$(Q_{10})^a$	T_{opt}(°C)[b]	T_{amb}(°C)[c]	$\rho_{T(opt)}$[d]	$\rho_{T(amb)}$[e]	$\rho_{T(amb)}/\rho_{T(opt)}$
	0.94	2.3					
$50T^2$	0.93		16	11	13.3	11.1	0.83
	0.96	2.1					
$^2 - 0.0042T^3$	0.96		19	11	27.3	20.4	0.76
	0.94	1.8					
$^2 - 0.0009T^3$	0.85		22	14	4.1	2.6	0.63
	0.95	2.3					
$^2 - 0.0021T^3$	0.95		23	14	11.6	6.9	0.59
	0.95	3.1					
$^2 - 0.0016T^3$	0.91		20	10	5.8	3.1	0.54
	0.93	2.8					
$31T^2$	0.88		22	10	13.3	8.5	0.64
	0.91	2.2					
$^2 - 0.0015T^3$	0.81		20	8	3.3	1.5	0.45
	0.97	2.4					
$^2 - 0.0025T^3$	0.95		21	8	8.5	2.3	0.27

fit of exponential to data over the range of positive response in ρ with increasing temperature.
squares fit of polynomial to entire data set.
us exchange of water with lake.
ynomial to entire data set.
water with lake.

TABLE 2. PAR, chlorophyll *a*, maximum transport rates at light saturation (ρ_L) and in the dark (ρ_D), ratios of dark to light-saturated maximum transport rates (ρ_D/ρ_L), and calculated kinetic parameters for experiments examining the light dependence of maximum DIN transport in Toolik Lake phytoplankton.

				ρ (nmol N·L^{-1}·d^{-1})						Kinetic parameters (NO_3^-)		
				NO_3^--N			NH_4^+-N				K_{LT}(±SD)	
Experiment	Date	PAR at 0.25 m (μE·m^{-2}·s^{-1})	Chl *a* (μg·L^{-1})	ρ_D	ρ_L	ρ_D/ρ_L	ρ_D	ρ_L	ρ_D/ρ_L	ρ_{max}(±SD) (nmol·L^{-1}·d^{-1})[a]	(μE·m^{-2}·s^{-1})[a]	(% PAR at 0.25 m)[a,b]
LT1	13 July 1980	111	1.7	4	70	0.06	53	184	0.29	89(6)	29(5)	26(5)
LT2	2 Aug. 1980	261	1.2	14	79	0.18	70	155	0.45			
LT3	23 Aug. 1980	189	1.5	3	44	0.07	46	112	0.41	55(8)	16(6)	8(3)
LT4	7 June 1981	152	4.0	3	62	0.05	45	144	0.32	71(6)	7(2)	5(1)
LT5	12 June 1981	282	1.2	6	27	0.22	61	121	0.50			
LT6	13 July 1981	124	2.1	37	119	0.31	113	217	0.52			
LT7	27 July 1981	232	1.2	28	118	0.24	113	197	0.57			
LT8	10 Aug. 1981	87	1.0	14	88	0.16	66	183	0.36			
LT9	15 Aug. 1981	91	1.2	9	95	0.09	53	196	0.27	102(9)	12(3)	13(3)

[a]Values of maximum light- and nitrate-saturated NO_3^- transport rates (ρ_{max}), half-saturation constants for PAR in maximum NO_3^- transport (K_{LT}), and associated standard deviations derived from least-squares fits of Michaelis–Menten relationship to data according to Cleland (1967).
[b]Values calculated as K_{LT} (μE·m^{-2}·s^{-1})/$I_{0.25m}$ (μE·m^{-2}·s^{-1}).

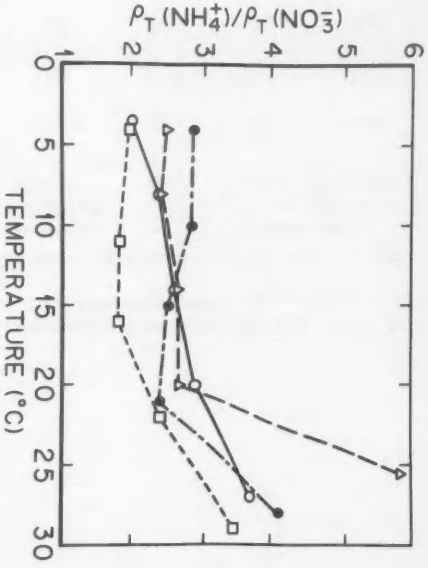

FIG. 2. Plots of maximum transport ratios, $\rho_T(NH_4^+)/\rho_T(NO_3^-)$, as a function of water temperature, Toolik Lake. □, T1; ○, T2; ●, T3; △, T4.

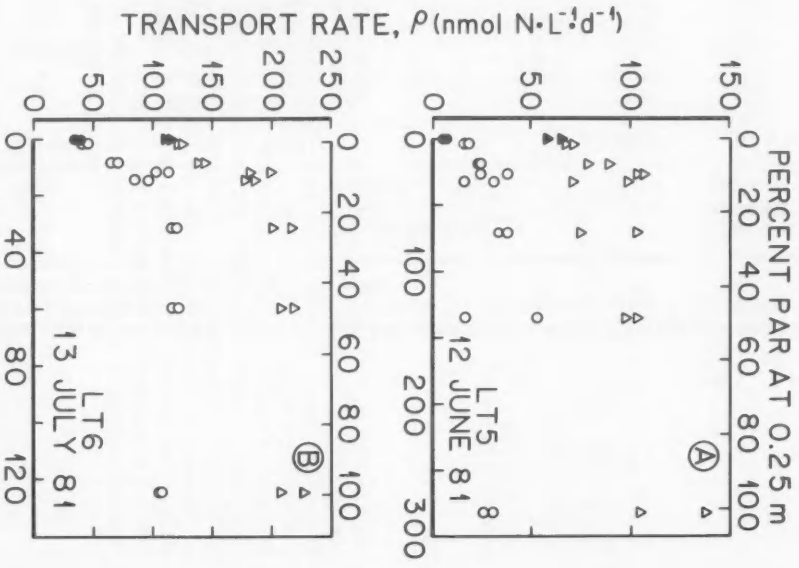

FIG. 3. Representative plots of the relationship between maximum NO_3^- (○) and NH_4^+ (△) transport rates and PAR, Toolik Lake. (A) Data suggesting loose coupling between DIN transport and PAR; (B) data seemingly described by a hyperbolic function. Solid symbols are DIN transport rates in the dark.

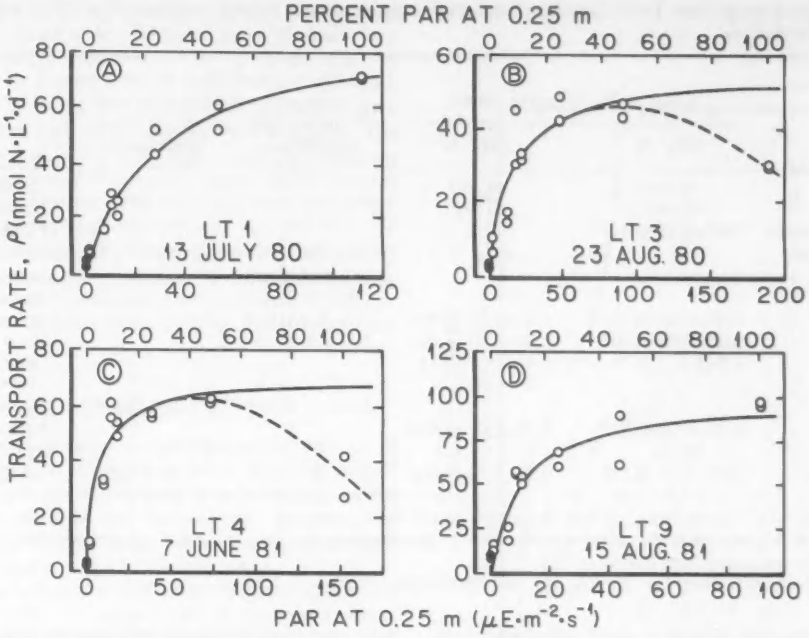

FIG. 4. Plots of maximum NO_3^- transport as a function of PAR, Toolik Lake. Solid curves are direct least-squares fits of rectangular hyperbolae to data according to equation (2). Broken curves in (B) and (C) are fit to data by eye. Solid symbols represent NO_3^- transport in dark (ignored in curve fitting).

four experiments but was never appropriate for the NH_4^+ transport data (Table 2). It should be noted, however, that a light effect was nonetheless evident for NH_4^+ transport in all experiments (cf. Fig. 3A, 3B) at approximately the same point (5–15% of PAR at 0.25 m) that the effect was observed for NO_3^- transport. Experimental data meeting the criteria of Nelson and Conway (1979) were directly fitted to equation (2) by the method of least-squares according to Cleland (1967) (Fig. 4), and kinetic parameters were calculated (Table 2). Depressed transport was noted in clear bottles in experiments LT3 and LT4 (Fig. 4B, 4C). This may have represented light inhibition, which has been reported in similar investigations (MacIsaac and Dugdale 1972; Murphy 1980). Therefore, in LT3 and LT4 the points from 100% PAR at 0.25 m were eliminated in curve fitting. Inclusion of these points in calculating K_{LT} and ρ_{max} led to the generation of curves obviously inadequate in describing the observed NO_3^- transport at low values of PAR. In the four experiments K_{LT} ranged from 7 to 29 $\mu E \cdot m^{-2} \cdot s^{-1}$ or from 5 to 26% of PAR at 0.25 m. The mean K_{LT} ($\bar{X} \pm$ SD) was 16 ± 9 $\mu E \cdot m^{-2} \cdot s^{-1}$ or 13 ± 9% of PAR at 0.25 m.

Discussion

Temperature Dependence Experiments

Published observations on the interaction of temperature and nitrogen-saturated transport of DIN in algae and macrophytes are few. Although the response curves are varied, all are in agreement with our data in that temperature limitation of DIN transport under conditions of substrate saturation was observed at the lower end of the temperature range examined. Kappers (1980) showed that from 13 to 19°C, maximum rates of NH_4^+ transport as a function of temperature in natural populations of Cyanobacteria followed a bell-shaped curve similar to the functions described in Fig. 1 for the entire range of temperatures examined. The optimum temperature for NH_4^+ transport by the Cyanobacteria was about 18°C. Ullrich et al. (1981) found that rates of nitrogen-saturated NO_3^- transport in *Lemna* sp. increased with temperature in the 10–30°C range, reached a single or double peak in the 30–35°C range depending on the degree of N-starvation, and decreased thereafter. Cloern (1977) showed that maximum rates of DIN transport in *Cryptomonas* sp. increased monotonically between approximately 5 and 30°C. In roughly the same temperature range, Rhee and Gotham (1981) reported a linear increase in the maximum rate of NO_3^- transport to 15°C in *Scenedesmus* sp. followed by a linear decline in transport with further temperature decreases.

The calculated optimum temperatures for nitrogen-saturated DIN transport by algae in Toolik Lake were well above the measured ambient water temperatures (Table 1) and were for the most part physically impossible to achieve under natural conditions. In fact, values of T_{opt} for maximum rates of DIN transport by these arctic populations of algae were similar to those cited above for a laboratory culture of *Scenedesmus* sp. and a natural population of Cyanobacteria in a temperate lake. However, these observations for N transport by algae in Toolik Lake are consistent with the data of Seaburg et al. (1981) for the temperature-*growth* response of Antarctic algal isolates. Four chlamydomonad clones showed optimum temperatures for growth well above the maximum in situ temperatures of their native habitats. The authors suggested that a key physiological advantage of these clones was their ability to grow reasonably well at cold temperatures and that they had evolved in warm environments but retained some temperature-growth

TABLE 3. Comparison of results from Toolik Lake light dependence experiments with literature values of $K_{LT}(NO_3^-)$ and ratios of V_D/V_L or ρ_D/ρ_L for nitrate and ammonium.

Region	ρ_D/ρ_L or V_D/V_L, range (mean)		$K_{LT}(NO_3^-)$		Reference
	NO_3^--N	NH_4^+-N	% surface light range (mean)	$\mu E \cdot m^{-2} \cdot s^{-1}$ range (mean)	
Temperate seas	(0.10)[a]	(0.26)[a]			Dugdale and Goering 1967
Tropical seas	(0.30)[a]	(0.59)[a]			Dugdale and Goering 1967
Czechoslovakian reservoirs	0.05–0.38 (0.11)[a]				Procházková et al. 1970
E. Tropical Pacific Ocean			14[b]		MacIsaac and Dugdale 1972
Peru Upwelling			0.9–13.3 (6.4)[b]		MacIsaac and Dugdale 1972
Northwest Africa			1.5–7.0 (5.4)[b]		MacIsaac et al. 1974
Baja, California	0.01–0.67 (0.16)	0.01–1.46 (0.49)	3.3–32.4 (16.1)		Nelson and Conway 1979
Northwest Africa	0.00–0.02 (0.007)	0.05–0.57 (0.36)	5.5–6.2 (5.9)		Nelson and Conway 1979
New York Bight	0.2–1.0 (0.7)	0.4–1.3 (0.7)			Conway and Whitledge 1979
Antarctic Ocean			1.1–2.3 (1.6)[b]	2.3–4.4 (3.1)[b,c]	Slawyk 1979
Lake Kinneret	0.16–0.91 (0.52)[a]	0.29–1.0 (0.65)[a]			McCarthy et al. 1982
Peru Upwelling	(0.69)				Dortch and Maske 1982
Toolik Lake	0.05–0.31 (0.15)	0.27–0.57 (0.41)	6–31 (15)[d]	7–29 (16)	This study

[a]Values calculated as $1/(V_L/V_D)$ or $1/(\rho_L/\rho_D)$ from reported values of V_L/V_D or ρ_L/ρ_D.
[b]Dark uptake ignored in fitting Michaelis–Menten relationship to light-nitrate uptake data, but was unreported percentage of V_L.
[c]Values calculated by converting from quanta $\cdot m^{-2} \cdot h^{-1}$.
[d]Values calculated from relationship $I_0 = 1.19(I_{0.25m})$; mean extinction coefficient = 0.7.

characteristics of ancestral strains in later colonizing cold environments. Thus, it is possible that algae indegenous to cold habitats are also selected in part for their ability to transport nutrients at cold temperatures with more relative efficiency than temperate species rather than for having an optimum temperature for transport closely allied with the ambient water temperature.

A feature common to all of the temperature dependence experiments was the rapid decrease in DIN transport at temperatures >25°C (Fig. 1). Similar declines in net and gross photosynthesis of phytoplankton cultures at supraoptimal temperatures have been respectively attributed to the accelerated increase in respiration relative to photosynthesis (Ahlgren 1978) and to the deterioration of the photosynthetic apparatus (Li 1980). Likewise, increased excretory loss, destruction of permeases, or impairment of metabolic capacity could account for the depressed DIN transport at supraoptimal temperatures. In addition, the possibility of "other limitation" during the period of incubation cannot be discounted. That is, severe phosphorus limitation could have occurred in bottles incubated at high temperature, resulting in reduced DIN transport. Similarly, Conway et al. (1976) and Dugdale et al. (1981) showed truncated uptake hyperbolas for limiting-nutrient uptake at the point where limitation shifted to another variable.

Values of the temperature coefficients ($\bar{X}$ = 2.4) for DIN transport by algae in Toolik Lake fall within the range of literature values reported for N and other nutrients. Tischner and Lorenzen (1981) gave Q_{10} values of 3.3 and 5.3 for NO_3^- transport in nitrogen-grown and nitrogen-starved *Chlorella* sp. cultures. From the data of Cloern (1977), Ullrich et al. (1981), and Rhee and Gotham (1981) we calculate Q_{10} values for DIN transport ranging from about 2 to 4. Dugdale (1976) cited temperature coefficients of 1.7 and 2.0 for silicate and phosphate transport in algae.

Light Dependence Experiments

Values of ρ_D/ρ_L or V_D/V_L (mathematically equivalent ratios) have often been calculated in field investigations of algal DIN utilization (Table 3) and have sometimes been used as a measure of the degree of light dependence in N-saturated DIN transport or uptake (e.g. Conway and Whitledge 1979; Nelson and Conway 1979). In all but the data of McCarthy et al. (1982) and Dugdale and Goering (1967), reported ratios (Table 3) were for N-saturated experiments. The literature values of V_D/V_L and ρ_D/ρ_L are highly variable and the range given in a particular study often varies by a factor of 3 or more. The range and mean values of ρ_D/ρ_L for NO_3^- in Toolik Lake are closest to the values given by Procházková et al. (1970) for productive Czechoslovakian reservoirs, while the range and mean for NH_4^+ are similar to the data of Nelson and Conway (1979) for the northwest African coast (Table 3). Except for the data from Lake Kinneret (McCarthy et al. 1982) and the New York Bight (Conway and Whitledge 1979), ρ_D/ρ_L is 2–5 times greater for NH_4^+ than for NO_3^-, indicating a greater light dependence of NO_3^- transport. This is in agreement with the proposed direct link (Falkowski and Stone 1975) between NO_3^- uptake and the photosynthetic process via the requirement for ATP from cyclic photophosphorylation for active NO_3^- transport across the plasmalemma.

The reason for the extreme variability in ρ_D/ρ_L for NO_3^- (Table 2) is unclear. It is possible that ρ_D/ρ_L for NO_3^- varies as a function of the degree of N-limitation in plankton populations. For example, Harrison (1976) reported enhanced dark NO_3^- transport in N-starved *Gonyaulax* sp. cultures relative to dark transport in N-sufficient cultures. In addition, Malone et al. (1975) found that NO_3^- transport in *Chaetoceros* sp. was independent of the light–dark cycle in N-limited cells but followed a diel pattern under nonlimiting conditions. However, no correlation was observed between ambient DIN levels in Toolik Lake and the ratio of ρ_D/ρ_L for NO_3^-, probably because DIN levels were uniformly low. The previous light history may have had some influence on ρ_D/ρ_L for both NO_3^- and NH_4^+, as low values tended to occur when experiments were conducted following a few days of overcast conditions.

Literature values of K_{LT} (% surface light) for maximum NO_3^- transport fluctuate widely, with the range of values for a given study varying in some cases by up to an order of magnitude (Table 3). Common to our study and that of Nelson and Conway (1979) is the fact that kinetic parameters were calculated only when dark NO_3^- transport was negligible. The Toolik Lake data for K_{LT} (% surface light: range and mean) are nearly identical with that of Nelson and Conway (1979) from the California coast. In absolute terms (microeinsteins per square metre per second), the range and mean of K_{LT} for NO_3^- in Toolik Lake were considerably higher than values reported by Slawyk (1979) for the Antarctic Ocean. However, dark uptake appears to have been substantial (we calculate V_D/V_L = 0.2–0.5) in the latter investigation, obviating direct comparison of data sets.

Extrapolation to Ambient Conditions

Our data demonstrate unequivocally that in the presence of transport-saturating concentrations of NO_3^- or NH_4^+, DIN transport in Toolik Lake phytoplankton is influenced by the individual effects of light and temperature. However, the question arises as to whether or not light and temperature limit DIN transport at the ambient levels of the lake.

Several field and laboratory studies have indicated that at low nutrient levels the effect of temperature on nitrogen transport and growth of algae is minimal. Eppley et al. (1979) found that although temperature and irradiance were significant independent variables , most of the variability in NH_4^+ transport by algae off the southern California coast could be explained in multiple regression analysis by variations in the ambient NH_4^+ concentration. Takahashi and Saijo (1981) noted that water temperature had little influence on the chlorophyll-specific transport rate ($\rho \cdot Chl^{-1}$) for NH_4^+ in Lake Kizaki, Japan, at substrate levels $<0.5\ \mu mol \cdot L^{-1}$. Morgan and Kalff (1979) found that growth in cultures of *Cryptomonas* sp. was strongly affected by light and temperature only when all nutrients were in excess. Rhee and Gotham (1981) concluded that the more severe the nutrient limitation, the greater the decrease in temperature necessary for temperature-controlled growth in *Scenedesmus* sp. and *Asterionella* sp. From these observations concerning the influence of temperature on growth and nutrient transport in algae we conclude that temperature was probably not a dominant factor controlling DIN transport by Toolik Lake phytoplankton at ambient nutrient levels (ΣDIN = $0.25\ \mu mol \cdot L^{-1}$). This is in spite of the demonstrated unused potential for nitrogen-saturated DIN transport at T_{amb} (Table 1).

At low ambient nutrient levels, MacIsaac and Dugdale (1972) demonstrated that NO_3^- uptake was nitrate-limited in the upper euphotic zone of the eastern tropical Pacific Ocean and light-limited in the lower euphotic zone. Furthermore, MacIsaac and Dugdale (1972) postulated that the crossover point between NO_3^- and light limitation of uptake occurred at the point where the in situ rate of uptake calculated from a known K_t (half-saturation constant for NO_3^- uptake) and V_{max} (maximum rate of NO_3^- uptake) and the Michaelis–Menten relationship was greater than the measured maximum or near-maximum rate of uptake at that depth. A typical chlorophyll-specific NO_3^- transport profile for Toolik Lake is shown in Fig. 5. Ambient NO_3^- levels were so low thoughout this and other depth profiles that limitation of $\rho \cdot Chl^{-1}$ was probably never achieved solely by light. Instead, the decreased chlorophyll-specific transport rate with depth must be interpreted in terms of the combined

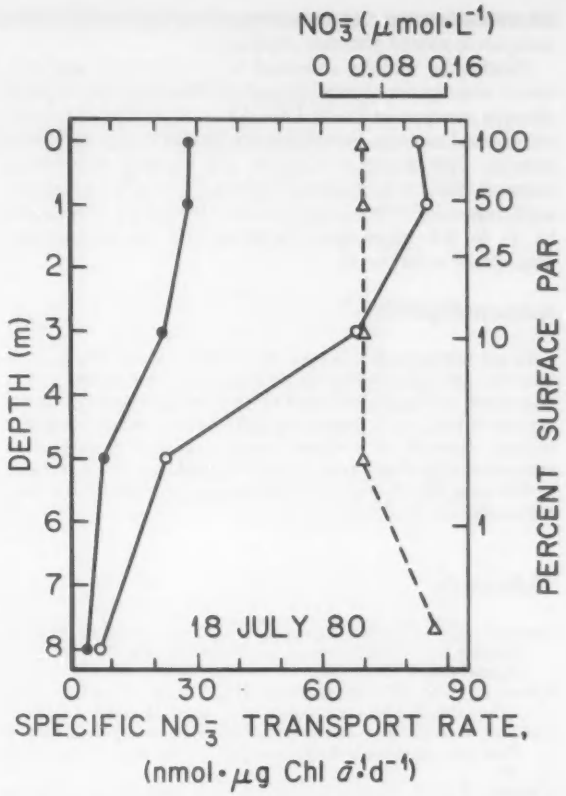

FIG. 5. Depth profile of chlorophyll-specific NO_3^- transport ($\rho \cdot Chl^{-1}$) and ambient NO_3^- concentration, Toolik Lake. ○, near-maximum chlorophyll-specific transport rates following addition of 3.07 μmol $^{15}NO_3^- \cdot L^{-1}$; ●, in situ rates calculated from Michaelis–Menten relationship (K_t = $0.12\ \mu mol \cdot L^{-1}$, $[\rho \cdot Chl^{-1}]_{max}$ calculated from observed near-maximum $\rho \cdot Chl^{-1}$); △, ambient NO_3^- concentration. Details of calculations given in MacIsaac and Dugdale (1972).

stress of NO_3^- limitation and suboptimal light. The calculated crossover point between NO_3^- and light limitation of transport at 8 m would occur at an NO_3^- concentration of $2.77\ \mu mol \cdot L^{-1}$, a value unrealized in the trophogenic zone of Toolik Lake. Therefore, we conclude that light also plays a secondary role in regulating DIN transport by Toolik Lake phytoplankton, the ambient concentration of DIN being of paramount importance.

Conclusions

Significant findings of our study are many: (1) We have extended the observation that maximum rates of DIN transport in algae are light-dependent from marine and temperate lacustrine systems to a high-latitude lake where light is continuous throughout the growing season. (2) We have broadened the well-documented observation that NH_4^+ is the preferred inorganic nitrogen source for algal nutrition to include arctic freshwater plankton as well. (3) Results of our study can be applied directly to predict phytoplankton response to unplanned perturbations through nutrient addition to arctic lakes, a distinct possibility as public access to the Alaskan arctic increases. (4) We have provided the first direct documentation concerning the

influence of temperature on nitrogen-saturated rates of DIN transport in natural plankton populations.

Clearly, the subjects addressed here are complex and continued investigation is needed to unravel the intricacies of algal nitrogen transport in Toolik Lake and to document its internal and external controls. Future research should attempt to directly examine light–nitrogen transport and temperature–nitrogen transport interactions at ambient lake levels of DIN. To this end, we recommend ^{15}N tracer additions of 0.05 $\mu mol \cdot L^{-1}$ followed by 1- to 3-h incubations for both light and temperature dependence experiments.

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Pacific Salmon (*Oncorhynchus*) Migrations: Orientation versus Random Movement

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Quinn, T. P., and C. Groot. 1984. Pacific salmon (*Oncorhynchus*) migrations: orientation versus random movement. Can. J. Fish. Aquat. Sci. 41: 1319–1324.

A computer simulation by Saila and Shappy (1963. J. Cons. Int. Explor. Mer 28: 153–166) indicated that the migrations of Pacific salmon (*Oncorhynchus*) from high seas feeding areas to the coastal vicinity of their natal stream may be accomplished with only slight homeward orientation. Five assumptions in this important paper are here reexamined to determine the validity of the original conclusion in light of current knowledge of Pacific salmon biology. It appears that assumptions concerning swimming speed, duration of migration, and return success were incorrect. These errors resulted in substantially underestimating the extent of salmon homeward orientation. Thus, contrary to the original conclusion, any hypothesis concerning the high seas migrations of salmon must explain the strong orientation of these fishes in the open ocean.

Selon une simulation sur ordinateur réalisée par Saila et Shappy (1963. J. Cons. Int. Explor. Mer 28: 153–166), les migrations de saumons du Pacifique (*Oncorhynchus*) à partir de zones d'alimentation en haute mer à des zones côtières proches de leur ruisseau natal peuvent s'accomplir avec seulement une faible orientation vers leur destination. On réévalue cinq hypothèses formulées dans cette importante étude afin de déterminer la validité des conclusions initiales en tenant compte des connaissances actuelles sur la biologie du saumon du Pacifique. Il semble que les hypothèses sur la vitesse de nage, la durée de la migration et le succès du retour soient incorrectes. Ces erreurs ont entraîné des sous-estimations substantielles de l'ampleur de l'orientation ves le ruisseau natal. Ainsi, contrairement à la conclusion originelle, toute hypothèse au sujet des migrations des saumons en haute mer doit expliquer la forte orientation de ces poissons dans l'océan.

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The return of Pacific salmon (*Oncorhynchus* spp.)across the open ocean to their spawning grounds constitutes one of the classic examples of animal migration. A number of experiments (reviewed by Hasler and Scholz 1983) have indicated that the selection of the salmon's home stream is made primarily on the basis of olfactory information experienced by the juvenile salmon years before. However, Hasler (1971) pointed out that the oceanic and riverine migrations are probably guided by different environmental cues.

Saila and Shappy (1963) attempted to assess the degree of orientation that salmon would need to return from the high seas to the coastal area where olfaction could take over as the guiding mechanism. They generated a Monte Carlo simulation of the movements of salmon from the North Pacific Ocean to the coast of North America, with swimming speed, distance and direction from home, and endurance as inputs. The simulated returns of salmon with no orientation at all were negligible, but only a relatively slight homeward orientation was required to simulate returns that approximated the observed returns of tagged salmon from the high seas to coastal regions. They further assumed that tag returns indicate homing success. Saila and Shappy concluded their paper by stating that they "... await the firm foundation of diverse biological data to support or modify our hypothesis."

Patten (1964) criticized the model and proposed another theoretical approach (involving searching patterns) to the problem of salmon homing behavior. Patten's conclusion, like that of Saila and Shappy, was that little orientation was needed for salmon to return from the open ocean. However, his model did not incorporate any biological data on salmon beyond that in the original simulation.

In the 20 yr since the publication of these analyses, our understanding of animal migration, orientation, and homing has increased considerably. Migrating birds and homing pigeons are known to use the sun, stars, and geomagnetic field for guidance (Keeton 1971; Wiltschko and Wiltschko 1978), and other sensory abilities may be used as well (Kreithen 1978). In addition to the compass orientation mechanisms that birds and other animals possess, there are indications that pigeons may also have a "map sense" or sense of their location, relative to their home (see discussions by Moore 1980; Gould 1982). Experimental studies have also documented solar and magnetic compass orientation in lake migrating juvenile sockeye salmon, *O. nerka* (Groot 1965; Brannon 1972; Quinn 1980; Quinn and Brannon 1982). However, literature reviews have generally concluded that there is no need to invoke map navigation to explain the high seas migrations of salmon (Harden Jones 1968; Leggett 1977; Able 1980). In these reviews, Saila and Shappy

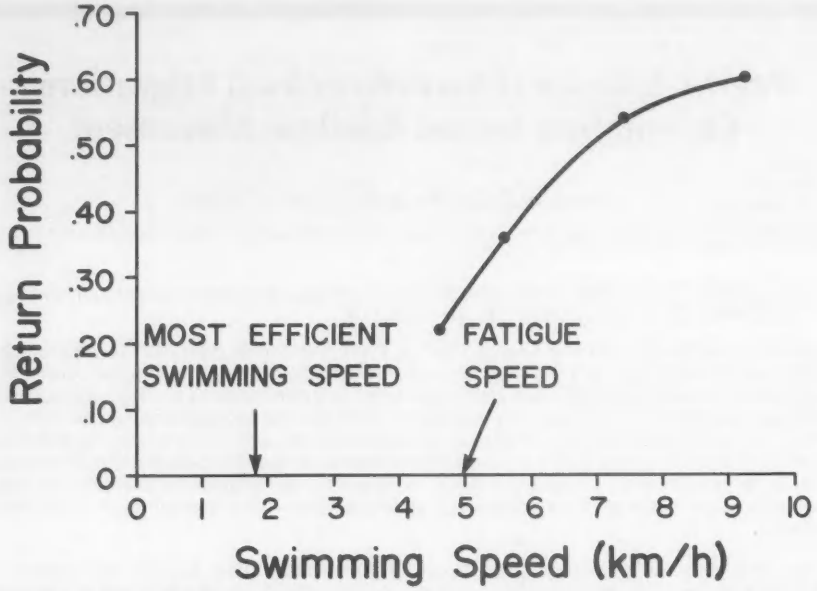

FIG. 1. Relationship between swimming speed and simulated homing success, at a given orientation strength ($A = 0.25$) (data from Saila and Shappy 1963 and Brett 1965a, 1983).

(1963) is cited as evidence (albeit indirect) that salmon migrations may have a substantial random component. The purpose of the present paper is to evaluate five crucial assumptions made in the simulation by Saila and Shappy (1963), in the light of current knowledge of salmon biology, and to review evidence relevant to the question of salmon orientation at sea.

Analysis of Assumptions

Swimming Speed

The importance of swimming speed in the simulation of homing success was demonstrated by Saila and Shappy (Fig. 1), who assumed that salmon swim 4.6 km/h at sea and 2.3 km/h in coastal waters. For a given orientation (homeward bias), homing success falls off markedly as swimming speed decreases. The swimming energetics of sockeye salmon have been studied in considerable detail since then, and Brett (1965a, 1983) indicated that the optimal (least cost) cruising speed of typical maturing sockeye salmon at sea (61 cm) is about 0.8 lengths/s or 1.8 km/h. Swimming is reasonably efficient up to 2.6 km/h, is inefficient at 4 km/h, and the salmon cannot sustain speeds over 5 km/h. Thus from an energetic standpoint, the assumed speed of 4.6 km/h seems too fast. Tracking studies of salmon in coastal waters indicate that they swim about 2 km/h (Madison et al. 1972; Stasko et al. 1976). At efficient speeds of 2–3 km/h, it is clear that virtually no salmon would return at the simulated level of orientation. Thus at slower, more realistic swimming speeds, more precise orientation is required to simulate return success.

High seas tagging studies revealed sockeye salmon travelling 40–60 km/d for several weeks from the high seas to coastal regions (Hartt 1966). Swimming at 2 km/h, salmon would have to be perfectly oriented ($A = 2.0$) to travel 48 km/d. Even at their maximum sustainable speed of 5 km/h, salmon would need an orientation of $A = 0.8$ (corresponding to $r = 0.4$ in the Rayleigh statistic commonly used in orientation research). Field evidence of orientation is not restricted to sockeye salmon, as Hartt (1966) reported average displacements of 50 km/d for pink salmon (*O. gorbuscha*) on the high seas. This is particularly interesting, as Brett (1982) reported that pink salmon can only sustain about 0.8 times the speeds of sockeye salmon, further evidence that a high degree of orientation at sea is required to explain the observed movements of tagged salmon.

Endurance (Duration of Migration)

Saila and Shappy permitted their simulated fish 175 d (25 wk) to reach the appropriate coastal area for homing, and termed this the limit of "endurance." Fish not reaching the appropriate coastal area in 175 d were considered to be lost. The term "endurance" is misleading in itself. It implies that salmon begin their homeward journey 175 d before they begin the ascent of their natal river system to the exclusion of other activities. According to present evidence, salmon are feeding and growing at sea with no indication of homeward movement much closer to the time of return. Sockeye salmon returning to Bristol Bay, Alaska, can be found over 3700 km of water in the east–west axis only 4–10 wk before they return home (Royce et al. 1968; Fig. 2A). Some of these salmon (identified by artificial tags or natural marks such as parasites or scale patterns) return from over 2200 km away. Thus the distinction must be made between salmon moving on the high seas while feeding, which they may do for several years, and maturing salmon leaving their immature cohort and migrating home. The fact that a salmon is at large for 175 d between capture at sea and recapture on the coast is not evidence that it spent 175 d attempting to return. On the contrary, there are a number of examples of salmon tagged at sea in late summer and recaptured 4–5 wk later at the mouths of rivers, having travelled long distances. Neave (1964) mentioned tagged sockeye that travelled about 1200 and 1600 km

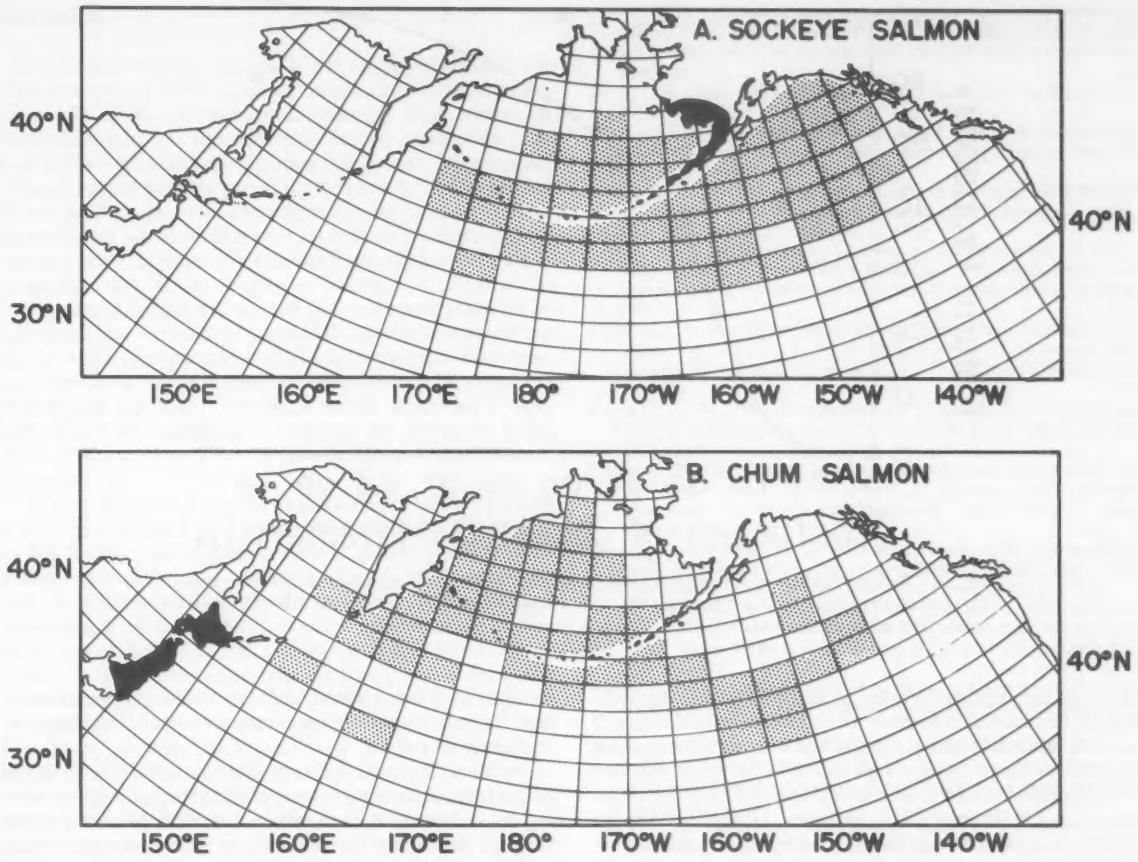

FIG. 2. (A) Distribution of maturing Bristol Bay sockeye salmon (data from French et al. 1976 and INPFC records). (B) Distribution of maturing Japanese chum salmon (data from Neave et al. 1976).

from open ocean to coastal areas in 27 and 35 d, respectively. These travel times would be accomplished if the fish continuously swam 1.85 and 1.90 km/h, respectively, on straight lines (discounting currents) for the entire journey. An even more dramatic case is that of a 42-cm sockeye tagged at about 153°W, 49.5°N on June 6, 1962, and recovered off the mouth of the Columbia River, 124°W, 46°N, on August 2 of that year (French et al. 1976). In 57 d, this small salmon travelled approximately 2200 km (1.60 km/h assuming a straight line). Interpolations from Brett's (1965b) data indicate that the optimal cruising speed of a 42-cm sockeye is about 1.0 length/s or 1.51 km/h, and the fatigue speed is 3.0 lengths/s or 4.5 km/h. It is clear from these examples that some salmon swim very long distances in brief periods of time, necessitating considerable orientation of some kind.

The assumption of a 175-d endurance period was erroneous because it assigned the salmon more travel time than they use. However, further evidence that the endurance period was inappropriate may be inferred from the precise timing of salmon returning to coastal areas. On average, 80% of the Bristol Bay sockeye salmon pass a narrow fishery in under 2 wk (Burgner 1980). Precise migratory timing characterizes many salmon populations and is important for reproductive success (Brannon 1984). Such timing is inconsistent with a high degree of random movement at sea.

Distance

Saila and Shappy placed their hypothetical fish 1200 nautical miles (2224 km) due west of their goal. As can be seen from the previous example, these conditions are not unprecedented. The oceanic distributions of salmon stocks can be quite broad, and not all fish will have to travel this far. However, maturing Japanese chum salmon (*O. keta*) may travel across virtually the entire North Pacific Ocean (up to 8500 km) to return home (Fig. 2B). Moreover, many salmon have to make complex migrations, such as Bristol Bay salmon feeding in the Gulf of Alaska that must swim around the Alaskan Peninsula (Royce et al. 1968; Fig. 2A). Overall, while the assumed distance and direction may not be typical, the application of these assumptions probably does not bias the model unduly.

Home Stream Recognition

The simulation assumed that any fish encountering the coast within 74 km of its home river was successful. The fact that the radius of recognition did not extend out to sea was noted by the authors but not considered important. Experiments (reviewed by Hasler and Scholz 1983) indicate that salmon have very sensitive olfactory systems. Unfortunately, there is no consensus as to the identity or nature of the chemicals that the salmon

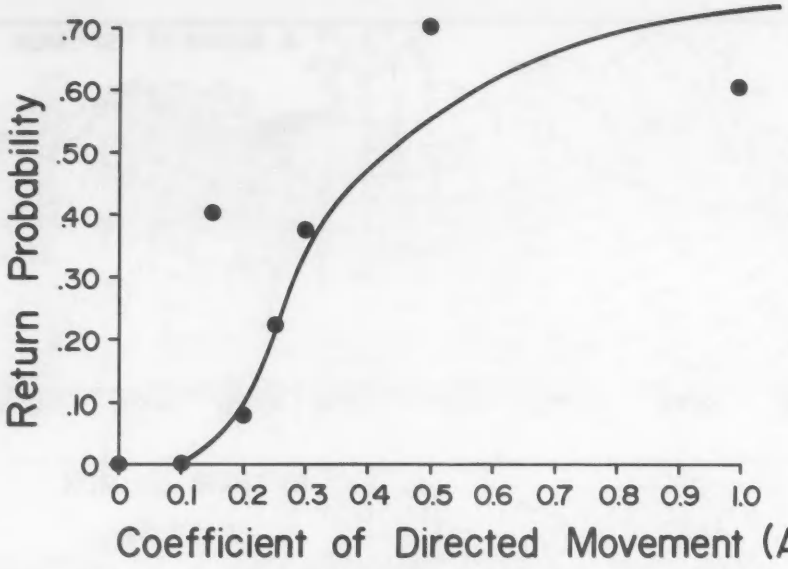

FIG. 3. Relationship between orientation strength and simulated homing success, at a swimming speed of 4.6 km/h (data from Saila and Shappy 1963, curve drawn by eye). For conversion to the Rayleigh r values commonly used in orientation analysis, $A = 2r$.

memorize and seek, and indeed the essential odor in one river may not be essential in another. Thus it might be misleading to calculate dilution factors and project how far an odorant could be detected. Large systems such as the Fraser River generate dilute domains that may extend several hundred kilometres from the river mouth (Favorite 1961). However, salmon also home to coastal streams that are blocked by sand bars during much of the fall (Shapovalov and Taft 1954; Heath 1960). However, the assumed 74-km radius of recognition is unlikely to have seriously biased the simulation, and there is no clear basis for an alternative figure, given the wide variety of salmon streams and the lack of knowledge about the olfactory imprinting process.

Proportion of Salmon Successfully Homing

The intuitive relationship between the proportion of salmon returning and the degree of orientation required to get them there was demonstrated by Saila and Shappy's simulation (Fig. 3). There was no explicit assumption of the actual proportion of salmon that home for comparison with the simulated returns. However, based on 10 and 22% returns of sockeye salmon tagged during International North Pacific Fisheries Commission operations, Saila and Shappy considered that 10–20% approximates the proportion of maturing salmon that successfully reach the coastal region for upstream migration. This is a seriously misleading assumption, as many factors may contribute to the failure to recover tagged fish. First, some predation at sea is known to occur (e.g. marine mammals: Fiscus 1980). Second, a small amount of tag loss probably occurs. Third, tagged fish may experience greater predation rates than untagged fish, due to the tag itself or to handling. The dangers of inferring orientation ability from tag returns are illustrated in data reported by Hartt (1962). Returns of chum salmon tagged south of Adak Island in the Aleutian chain varied 20-fold according to the type of tag (disc or tube) and size of salmon (greater or less than 45 cm). This difference can scarcely be attributed to orientation ability.

Predation, tag loss, and mortality account for some of the tagged fish that were not recovered. However, two other factors are also important. Tagged salmon that reach their natal stream to spawn and die are almost certain to do so undetected. There are thousands of salmon streams in Asia and North America and relatively few are surveyed in any but the most cursory manner. In 1982, 20 804 sockeye were tagged at the mouth of Adams River in Shuswap Lake, British Columbia. In spite of intensive search in the main river and adjacent spawning areas, only 2940 of the tagged salmon (14%) were recovered (International Pacific Salmon Fisheries Commission, unpubl. data). The loss of 86% of the tags in this densely populated but closely monitored system indicates how easy it is for tagged fish to spawn undetected if they escape fishermen. The other major source of tag loss is the failure of fishermen to report marked fish. Some legal fishermen make little effort to return tags, and no tags will be returned by the all-too-numerous poachers. A recent study revealed a tag-reporting rate of only 29% from marine recreational fishermen in Texas (Green et al. 1983), giving reason to believe that failure to report tags may be very common among salmon fishermen as well. Thus there are many reasons why all salmon tagged at sea are not recovered in coastal areas.

All things considered, it is surprising that as many as 20% are recovered. Far from being evidence that 80% of the maturing salmon are lost at sea due to disorientation, this figure is entirely consistent with a very small proportion of lost fish. Additionally, if salmon movements at sea and in coastal areas are largely random, we would expect that substantial numbers of salmon would spawn outside their natal river rather than die without spawning. On the contrary, remarkably few salmon spawn long distances from their home river (e.g. Quinn and Fresh 1984).

Discussion

Based on their simulation, Saila and Shappy concluded that while salmon swimming at random would virtually never locate the mouth of their natal stream, relatively little orientation (homeward bias) was needed to simulate the results of tagging studies. Two assumptions, regarding the distance and direction of travel required and the proximity to the home stream needed for recognition, are broad generalizations but probably did not seriously bias the study. However, as indicated in the foregoing analysis, errors in three very important assumptions resulted in underestimation of the degree of orientation required for homing: swimming speed was too fast, endurance (duration of migration) was too long and ignored the precision of migratory timing, and the proportion of adult salmon successfully returning home from the high seas for comparison with simulated returns was too low. Overall, it would seem that a very high degree of orientation is required for salmon to cover 1000–2000 km in 4–10 wk, a feat that tagging studies have demonstrated.

The mechanisms of oceanic orientation are still a matter of controversy. Leggett (1977) reviewed a large body of literature on fish migration and concluded that the most likely mechanism underlying long distance seasonal migrations is the maximization of physiologically optimal conditions. While compass orientation is not discounted, it is not of paramount importance in this model. An alternative, proposed by Quinn (1982), is that salmon have a bicoordinate map allowing them to select the appropriate compass direction for homing, and then maintain this direction with a magnetic or celestial compass. Both hypotheses presently lack conclusive supporting evidence. However, any proposed homing mechanism must be consistent with the movement of salmon for 4–10 wk at average rates of 40–60 km/d (Hartt 1966), based on straight line travel. Salmon cannot sustain speeds greater than about 5 km/h, and all evidence indicates that they actually swim about 2 km/h, near their most efficient speed. Salmon of a given population, converging on a precise coastal area from a broad oceanic distribution, experience a wide range of environmental conditions (Neave 1964; Royce et al. 1968). All available evidence indicates that very few salmon are lost due to disorientation at sea. The great majority of the surviving salmon return to the vicinity of their natal river over a short period of time. In summary, current information is inconsistent with a model of salmon migration based on largely random searching behavior, though the guidance mechanism is not yet understood.

Acknowledgments

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Generalized Stock Reduction Analysis

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Kimura, D. K., J. W. Balsiger, and D. H. Ito. 1984. Generalized Stock Reduction Analysis. Can. J. Fish. Aquat. Sci. 41: 1325–1333.

In this paper we generalize Stock Reduction Analysis (SRA) so that the model now allows for growth in the fishable biomass, and variable recruitment to the fishable biomass. The application of R. B. Deriso's delay-difference equation to SRA allows for growth, and also provides justification for using a biomass based model. Variable recruitment is introduced into SRA using both a stock–recruitment relationship and exogenous variables. We present general methods for obtaining unique solutions to the SRA equations, and give a new method for calculating the equilibrium sustainable yield curve. By applying the generalized SRA model to Pacific ocean perch (*Sebastes alutus*) stocks in the Gulf of Alaska, we conclude that the sustainable yield from this stock is smaller than previously estimated.

Les auteurs généralisent l'analyse de réduction des stocks (ARS) de sorte que le modèle tient compte d'une augmentation de la biomasse exploitable et d'un recrutement variable relatif à cette biomasse. L'application de l'equation de variation en différé de R. B. Deriso à l'ARS permet de tenir compte de la croissance et justifie aussi l'utilisation d'un modèle basé sur la biomasse. L'introduction d'un recrutement variable dans l'ARS est effectuée à l'aide d'une relation stock–recrutement et de variables exogènes. Les auteurs présentent des méthodes générales pour obtenir une seule solution à l'équation de l'ARS ainsi qu'une nouvelle méthode de calcul de la courbe d'équilibre du rendement soutenu. L'application du modèle généralisé d'ARS aux stocks de sébaste à longue mâchoire (*Sebastes alutus*) du golfe d'Alaska permet de conclure que le rendement soutenu de ces stocks est moins élevé qu'on l'avait estimé.

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Stock reduction analysis (SRA) is a biomass based method of stock assessment that uses the exponential form of the catch equations and annual catches recorded in weight. Given n years of catch data, the original SRA equations (Kimura and Tagart 1982) constitute a system of $n + 1$ equations in $n + 4$ unknowns ($F_1,\ldots, F_n, M, B_1, P, R_0$; see Appendix A for definitions). Thus these equations do not have a unique solution without additional constraints. SRA is a systematic way of finding the most reasonable solutions to these equations.

The original SRA model (Kimura and Tagart 1982) suffered from two notable deficiencies. First, growth did not appear explicitly in the model. This caused growth to be lumped with recruitment, which resulted in a nonstandard treatment of both growth and recruitment. In fisheries modeling, it is customary for "growth" to refer to the growth of a biomass already recruited to the fishable stock, and for "recruitment" to refer to the recruitment of new fish into the fishable stock. A second deficiency was that the original SRA model assumed constant recruitment (defined as new fish plus growth in the fishable stock). It is generally accepted that many, if not most, marine fish species exhibit large variations in recruitment. In this paper we generalize the original SRA model in an attempt to overcome these deficiencies.

Our most important innovation is to use Deriso's delay-difference equation (Deriso 1980) to describe the interannual changes in population biomass. Deriso (1980) has shown that if recruitment is knife-edged, and growth of individuals in a population is governed by the Brody weight equation, then the population biomass at the beginning of any year is a function of the population biomass at the beginning of the two previous years plus the recruitment biomass of new fish. The delay-difference equation not only introduces growth into the SRA analysis (thereby correcting the previously noted deficiencies in the operational definitions of growth and recruitment), but also provides a theoretical justification for using a biomass based model.

Accounting for variable recruitment in the SRA model is more difficult because SRA is a deterministic way of solving the catch equations, whereas variable recruitment is conceptually a stochastic phenomenon. We introduce variable recruitment into the SRA model in two ways. The first, which we call "Cushing recruitment," assumes a stock–recruitment relationship. The second, which we call "proportional recruitment," assumes that recruitment indices are available from ancillary data and that recruitment is proportional to these indices.

Generalized Stock Reduction Analysis Model

A list of definitions of variables used in this paper can be found in Appendix A. As in the original SRA, consider the sequence of $i = 1,\ldots, n$ catch equations:

$$(1) \quad C_i = B_i F_i (1 - s_i)/Z_i.$$

B_i is the population biomass at the beginning of year i and, in

general,

$$(2) \quad B_i = (1 + \rho)s_{i-1}B_{i-1} - \rho s_{i-1}s_{i-2}B_{i-2} + R_i \qquad \text{for } 2 \leq i \leq n + 1.$$

To this set of $2n$ equations (equations 1 and 2), add the equation

$$(3) \quad P = B_{n+1}/B_1$$

which describes the relative change in population biomass associated with the n years of catches.

Equations 1 and 3 are identical to the equations used in the original SRA (Kimura and Tagart 1982). Equation 2 is the Deriso delay-difference equation (Deriso 1980), which describes the interannual change in population biomass. Because SRA requires that B_2 be a function of B_1 only, but equation 2 indicates that B_2 is a function of B_1 and B_0, we redefine B_2 as a function of B_1. We assume that the biomasses at the beginning of year 1 and year 0 are the same (i.e. $B_1 = B_0$), so equation 2 can be rewritten as

$$(4) \quad B_2 = B_1 s_1(1 + \rho - \rho s_0) + R_2 \qquad \text{for } i = 2 \text{ only.}$$

If B_1 is a virgin biomass, $s_0 = \exp(-M)$. However, if B_1 is not a virgin biomass, s_0 must be estimated from some other source.

Variable recruitment will be introduced into equation 2 (and 4) in two different ways. In the first, we assume a stock–recruitment relationship of the form

$$(5) \quad R_i = R_1(B_{i-k}/B_1)^r \qquad i > k.$$

In this equation, R_1 is the recruitment expected from stock size B_1 (or when $i \leq k$), $r \geq 0$ is a constant describing the strength of the stock–recruitment relationship, and k is the age at recruitment. Cushing (1971) extensively applied this stock–recruitment relationship, so we refer to it as Cushing recruitment. If $r = 0$ in Cushing recruitment, we then have a constant recruitment model.

Our second method of modeling variable recruitment assumes that we have indices p_i ($0 \leq p_i \leq 1$, $i = 2,\ldots, n + 1$) of relative annual recruitment strength. These indices must be available from other sources such as surveys, environmental variables known to be related to recruitment, or other modeling efforts. The peak recruitment year should be assigned a p_i value of 1, and all other indices standardized to this value. We then assume

$$(6) \quad R_i = R_{\max} p_i.$$

Here, $R_{\max}$ is the peak recruitment and the p_i are known constants. This form of variable recruitment, which we call proportional recruitment, does not assume a stock–recruitment relationship.

Equations 1–6 fully define the generalized SRA models that we will use in this paper. It follows from equation 2 that when the Brody weight coefficient is set equal to zero ($\rho = 0$), and recruitment is made constant ($R_i = R_0$), then equations 1–3 are identical to the original SRA model.

Solving the General SRA Equations

As in the original SRA model, we have $2n + 1$ simultaneous equations (equations 1–3). If we assume prior estimates of M, ρ, r and p_i, these $2n + 1$ simultaneous equations are a function of $2n + 3$ unknowns ($B_1,\ldots, B_{n+1}$, R, P, $F_1,\ldots, F_n$). Here, R denotes either R_1 or $R_{\max}$, the recruitment parameter to be estimated from SRA.

Given an estimate of B_1, the unknowns B_i ($i > 1$) can be estimated from forward calculations using equation 2. Therefore, equations 1 and 3 can be viewed as a set of $n + 1$ simultaneous equations in $n + 3$ unknowns (B_1, R, P, $F_1,\ldots, F_n$). Hence there is no unique solution to equations 1 and 3; any $n + 3$-tuple that satisfies the $n + 1$ simultaneous equations is consistent with those equations.

It is tempting to state that if any two of the variables (B_1, R, P, $F_1,\ldots, F_n$) are known, equations 1 and 3 can be solved for the remaining $n + 1$ unknowns, but this is not quite true. If one attempts to fix B_1 and F_1, the resulting equation would be inconsistent, since B_1 determines F_1 through equation 1. We do not know if there are other potential inconsistencies hidden in equations 1–4. Nevertheless, generally speaking, if two allowable quantities ("allowable" meaning allowing solutions) among (B_1, R, P, $F_1,\ldots, F_n$) are known, equations 1 and 3 can be solved for the remaining $n + 1$. We do not think the kind of inconsistency discussed here is a serious limitation to SRA, and it does not appear to be a problem in the solution methods we now describe.

Among the variables (B_1, R, P, $F_1,\ldots, F_n$), B_1 and R seem to be most closely related to the population's biology, whereas (P, $F_1,\ldots, F_n$) seem more related to fishing. Therefore our analyses emphasize the B_1 versus R plane. One reason the B_1 versus R plane is conceptually useful is that there exists an isomorphism (i.e. a one-to-one relationship) between allowable values of (B_1, R) and solution points (B_1, R, P, $F_1,\ldots, F_n$). This can easily be proved from the fact that given an allowable value of (B_1, R), then (P, $F_1,\ldots, F_n$) is always uniquely determined by stepping through equations 1–4. Thus an allowable value of (B_1, R) can be thought of as a complete solution (i.e. an $n + 3$-tuple) to equations 1 and 3.

With this in mind, plots in the B_1 versus R plane, which we call SRA plots, become our principal conceptual tool for examining solutions to the SRA equations. Since each allowable (B_1, R) point represents a unique solution to the SRA equations, our goal is to narrow the solution set to a single point on an SRA plot. If we condition on (i.e. fix) any one of the variables (B_1, R, P, $F_1,\ldots, F_n$), the resulting solution set can be represented by a curve or line in the B_1 versus R plane. If two of these curves (or lines) intersect, their point of intersection represents a unique solution to the SRA equations.

To determine a unique solution then, we need additional information on two allowable variables among (B_1, R, P, $F_1,\ldots, F_n$). Possible sources of information include survey results, changes in catch per unit effort in the commercial fleet, or results from computer modeling (e.g. Virtual Population Analysis (VPA)). Typical SRA plots may show a range in the variables for which data are available, so that one can get a feeling for the probable range in the solution set. The exact nature of the SRA plot will depend on the goal of the assessment scientist and the data available.

We have shown that two allowable variables among (B_1, R, P, $F_1,\ldots, F_n$) must be fixed in order to have a unique solution to the SRA equations. By making the additional assumption that B_1 is a virgin biomass, it is possible to draw an additional line on the SRA plot. This means that a unique solution to the SRA equations can then be obtained by knowing only one of the variables (B_1, R, P, $F_1,\ldots, F_n$). To accomplish this, we introduce the concept of an expected recruitment line (ERL).

We must now restrict our discussion to the SRA model defined by equations 1–4 with Cushing recruitment (equation 5). The concepts of equilibria, virgin biomass, and ERL are not applicable to SRA models with proportional recruitment (equation 6) because populations governed by exogenous

variations in recruitment are constantly cycling, never attaining an equilibrium.

If B_1 is a virgin biomass and recruitment is constant, we can assume that B_1 is in equilibrium with natural mortality, growth, and recruitment. Simply stated from equation 2, at equilibrium we would expect the relationship between B_1 and R_1 to be

$$B_1 = (1 + \rho)\exp(-M)B_1 - \rho\exp(-2M)B_1 + R_1.$$

Solving for R_1, we have the ERL

$$(7) \quad R_1 = B_1\{[1 - \exp(-M)] + \rho[\exp(-2M) - \exp(-M)]\}.$$

When the Brody weight coefficient is equal to zero ($\rho = 0$), equation 7 is the same ERL used in the original SRA.

The ERL is applied by drawing equation 7 on an SRA plot. Now, only combinations of B_1 and R_1 that fall on this line are consistent with the assumption that B_1 is a virgin biomass. It can now be seen that given only one of the variables (B_1, R, P, $F_1,\ldots,F_n$), a unique solution to the SRA equations is determined.

It is important to realize that the ERL cannot be applied when B_1 is not a virgin biomass, or when recruitment is driven by exogenous variables (equation 6).

Once a unique solution ($\hat{B}_1$, $\hat{R}$, $\hat{P}$, $\hat{F}_1,\ldots,\hat{F}_n$) to the SRA equations has been obtained, equations 1–4 can be used to estimate a time series of biomass, exploitation rate, and recruitment.

We have not by any means exhausted the possible ways of solving the SRA equations. Given the proper data, conceivably ρ, r, or even M could be estimated using SRA. Also, depending on the circumstances, plots other than B_1 versus R may be desired.

Computer Solutions to the SRA Equations

As with the original SRA model, solutions to the generalized SRA model can be calculated using ZSYSTM (IMSL, Inc. 1979). ZSYSTM is a Fortran callable subroutine designed for finding iterative solutions to general systems of m simultaneous nonlinear equations in m unknowns. Most software designed to perform this task should work.

Since we have $n + 1$ simultaneous equations (equations 1 and 3), ZSYSTM requires that we fix two allowable variables among (B_1, R, P, $F_1,\ldots,F_n$). We assume that all other parameters in the SRA model (i.e. M, ρ, r, p_i) are held constant. To generate SRA plots, a convenient strategy is to write a program to fix B_1, and any one of the other variables P, $F_2,\ldots,F_{n-1}$ or F_n. The two initially fixed values plus the $n + 1$ solution values obtained from ZSYSTM constitute a solution to the SRA equations. A curve on an SRA plot is generated by varying B_1 while keeping the second conditioning variable fixed.

We recommend that SRA programs be interactive because this allows an immediate retrial using new initial values should the iterative process fail. $P = 1$ and $F_i = 0$ seem to be adequate initial values for these variables, but the initial R value is more critical to the iterative process and should be input interactively.

Besides the general SRA program we have described, the user should have a simpler SRA program that does nothing more than accept fixed values of (B_1, R) and solves equations 1–4 in the forward direction. This accomplishes several things. First, it provides a way of verifying any solution obtained from the more complicated SRA program. Second, it provides the complete solution associated with any allowable value of (B_1, R). And finally, by setting R from the ERL (equation 7), it allows the calculation of SRA solutions that are restricted to the ERL.

Whenever we are trying to find solutions to the SRA equations in a region where P is small, the general iterative program may fail because no solution exists, or because catch exceeds biomass for some intermediate iteration of parameters. Since there are no intermediate solutions, the only way the simplified program can fail is when B_1 and R are so small that catches exceed biomass at the beginning of some year. Solutions restricted to the ERL are especially useful when P is believed to be small. It then becomes easier to find the desired solution by moving along the ERL.

Generally, if we are restricting our solutions to the ERL, the F_i and P solutions will be monotonic functions of B_1. Therefore, the simplified SRA program should be sufficient for finding nearly any desired solution. This means that when the ERL is being used, SRA can be applied without a general nonlinear equation solver. However, even in the simpler SRA program, an iterative algorithm is needed to solve equation 1 for F_i.

SRA uses solutions from systems of simultaneous nonlinear equations. This contrasts with many nonlinear stock assessment models that are fit using nonlinear least squares or maximum likelihood. For these models, usually a general iterative program is used that cannot guarantee that a global minimum (or maximum) has been achieved. In the worst cases such programs sometimes seem to do little more than feed back initial input values, now accepted as solutions. The SRA method avoids this problem. Any questionable solution to the SRA equations can be easily verified.

Parameters Required Prior to SRA

In the previous sections we assumed that all parameters other than (B_1, R, P, $F_1,\ldots,F_n$) were known prior to performing SRA. In this section we discuss those other parameters that must be estimated (or at least fixed) before an SRA stock assessment can be performed. These include the instantaneous natural mortality rate M and the Brody weight coefficient ρ. If the Cushing recruitment model (equation 5) is used, values for r and k are required; if the proportional recruitment model (equation 6) is used, estimates of the p_i are required. An important characteristic of SRA is that it does not require age data. However, age data are indirectly required in that estimates of M and ρ are needed.

The estimation of M is one of the more thoroughly studied problems in fisheries. This problem is reviewed by Ricker (1975), and discussed from a statistical point of view by Chapman and Robson (1960). Despite the limited accuracy of most estimates of M, a stock assessment scientist can generally find estimates of M in the literature for the species he is studying, or for a closely related species.

A discussion of how the Brody weight coefficient can be calculated is given in Appendix B. Required data include mean weight at age and the age at recruitment. Recalling that the generalized SRA model with Cushing recruitment is equivalent to the original SRA model when $\rho = 0$ and $r = 0$, one can hope that SRA would be fairly robust to poor estimates of ρ. When ρ is underestimated as in the original SRA, we can assume that the missing growth component of fishable biomass is being lumped with recruitment. The biases introduced by overestimating ρ are less clear and may be more serious.

Estimates of parameters related to variable recruitment are

more difficult to come by. For Cushing recruitment, r describes the strength of the stock–recruitment relationship (Fig. 1). When $r = 0$, Cushing recruitment is a constant recruitment model and the population is highly resilient. When $r = 1$, recruitment is proportional to biomass and the population has no resilience. If fishing occurs on such a population, the population is reduced and remains in equilibrium at the lower level even after fishing ceases. Thus for all populations we can assume $0 \leq r \leq 1$. When applying Cushing recruitment, the researcher has a further choice of applying a stock–recruitment lag of k years.

Under ideal circumstances data may be available to estimate r in the Cushing recruitment model. However, it is more likely that the researcher would simply hypothesize a value for r in order to examine what effects a stock–recruitment relationship has on his assessment. An estimate of k can come from the perceived age at recruitment (see Appendix B). If the stock is depleted (i.e. $P \ll 1$, it is likely that recruitment has been reduced, and $r > 0$ may be an appropriate assumption for an assessment.

The proportional recruitment model requires estimates of p_i for $i = 2,\ldots, n + 1$. These parameters may be estimated from a number of sources. Possible sources include survey indices of relative recruitment strength, estimates of environmental factors known to affect recruitment strength, or modeling results (perhaps from VPA) that indicate relative recruitment strength.

Because of the large number of parameters required for a proportional recruitment SRA, and because the ERL cannot be applied to this form of the SRA model, it appears that assessments based on this model are the most difficult to perform. To obtain a unique solution to the SRA equations, we require estimates of two allowable variables among (B_1, R, P, $F_1,\ldots, F_n$).

Estimates of Sustainable Yield

Once we have solutions to the Cushing recruitment form of the SRA model, we can estimate equilibrium biomass (B) and equilibrium yield (Y) for a given fixed instantaneous fishing mortality rate F. Let $Z = F + M$, and $s = \exp(-Z)$. At equilibrium, equation 2 with Cushing recruitment can be written as

$$B = (1 + \rho)sB - \rho s^2 B + R_1(B/B_1)^r.$$

Solving for the equilibrium value of B,

$$(8) \quad B = \{(R_1/B_1{}^r)/[1 + \rho s^2 - (1 + \rho)s]\}^{\frac{1}{1-r}}.$$

Since the exploitation rate is

$$U = F(1 - s)/Z,$$

the equilibrium yield is

$$(9) \quad Y = UB.$$

Equations 8 and 9 do not depend on B_1 being a virgin biomass. In fact, virgin biomass can be estimated from equation 8 when $F = 0$.

For $r = 1$ there is no sustainable level of fishing. Any fishing continually diminishes the population until it reaches extinction. If such a population is fished down, and then fishing ceases, the population will remain at the reduced level, which is now a new equilibrium.

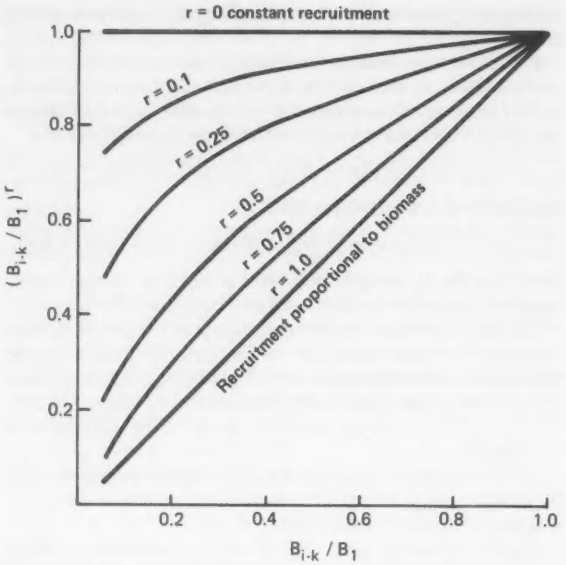

FIG. 1. Stock–recruitment relationship for the Cushing recruitment model. The relationship between stock size and recruitment is $R_i = R_1(B_{i-k}/B_1)^r$.

The relationship between F and Y can be parabolic or monotonic depending on the value of the parameters. Estimates of MSY can be obtained from the peak of a parabolic curve, or from the value of Y when $F = M$ (Gulland 1970; Francis 1974).

Application of SRA to Pacific Ocean Perch (*Sebastes alutus*) in the Gulf of Alaska

Preliminary Information

The fishery for Pacific ocean perch (*Sebastes alutus*) in the Gulf of Alaska has been historically significant. Catches from 1961 to 1976 (Table 1) made exclusively by foreign fleets averaged 106 637 t/yr. Pacific ocean perch is a rather slow-growing, low-fecundity species that can reasonably be expected to possess a stock–recruitment relationship. Therefore this population appears to be a good candidate for assessment with generalized SRA using the Cushing recruitment model. We hope to accomplish two goals: first, show how to apply the generalized SRA model, and second, demonstrate that present population estimates of maximum sustainable yield (MSY) and equilibrium yield (EY) may be significantly overestimated for this population.

A controversy currently exists over the proper age determination methodology for Pacific ocean perch. Broken and burned otolith advocates have determined maximum ages of over 70 yr (Beamish 1979). Surface otolith ages tend to be significantly lower (for ages greater than 16 yr) and a natural mortality rate of $M = 0.15$ appears to be consistent with these lower ages (see Ito 1982). We shall use $M = 0.15$ so that our assessment can be compared with previous assessments without being confounded by the ageing controversy.

To estimate the Brody weight coefficient ρ, we need an estimate of the age at recruitment k and average weight at age (Appendix B). Catch at age data from Ito (1982) show that from

TABLE 1. Annual catch of Pacific ocean perch in the Gulf of Alaska (adapted from Ito 1982).

Year	Catch (t)
1961	16 000
1962	65 000
1963	136 300
1964	243 400
1965	348 600
1966	200 800
1967	120 000
1968	100 200
1969	72 600
1970	44 900
1971	77 500
1972	77 600
1973	56 400
1974	51 000
1975	50 400
1976	45 500

TABLE 2. Average weight at age for Pacific ocean perch in Queen Charlotte Sound (adapted from Gunderson 1977).

Age (yr)	Weight (kg)
9	0.573
10	0.662
11	0.741
12	0.826
13	0.903
14	0.977
15	1.040
16	1.105
17	1.164
18	1.225
19	1.270
20	1.316
21	1.354
22	1.392
23	1.431
24	1.461

1961 to 1976, the average annual modal age in the catch was 9.7 yr. From this we determine the age at recruitment to be $k = 9$ yr. Using $k = 9$, and the average weight at age data (Table 2) for Pacific ocean perch from Queen Charlotte Sound, nonlinear least squares (Appendix B, equation 12) can be used to estimate $\rho = 0.5$.

Assuming that the population biomass at the beginning of 1961 is a virgin biomass, the ERL can be applied. The main ancillary information we hang our SRA analysis on is that survey results from 1961 to 1976 show an enormous decline in catch per unit effort (Table 3). We interpret this to mean that P is necessarily small in acceptable SRA solutions.

Summarizing, we have $M = 0.15$, $k = 9$, $\rho = 0.5$, and $P \ll 1$. The critical variable r in the Cushing recruitment model will be explored in our SRA assessment.

Stock Assessment Using SRA

An SRA plot for Pacific ocean perch in the Gulf of Alaska is shown in Fig. 2. Assuming that B_1 at the beginning of 1961 is a

TABLE 3. Average catch per unit of effort (CPUE) from trawl surveys for Pacific ocean perch in the Gulf of Alaska, 1961 and 1973–76, by region and depth zone (adapted from Shippen and Stark 1983).

	1961 CPUE (kg/h)		1973–76 CPUE (kg/h)	
Region	101–200 m	201–400 m	101–200 m	201–400 m
Fairweather	7.4	149.7	2.9	0.0
Yakutat	66.3	85.0	4.6	6.2
Prince William	48.8	80.1	10.9	1.5
Kenai	80.4	31.8	4.7	0.0
Kodiak	25.2	2.9	2.4	11.7
Shelikof	2.4	10.5	1.4	1.0
Chirikof	135.0	67.8	18.3	0.4
Shumagin	29.3	431.4	—	—
Sanak	7.2	228.6	0.0	53.5
Total	50.1	75.6	6.1	3.4

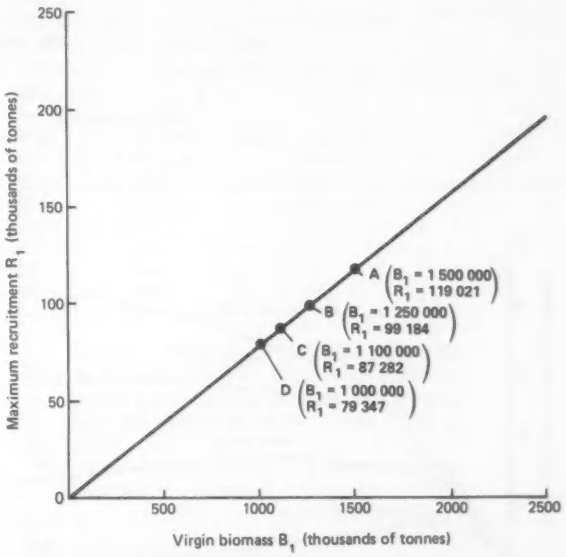

FIG. 2. SRA plot for Pacific ocean perch in the Gulf of Alaska. Points A, B, C, and D represent different virgin biomass and recruitment values that are considered in our assessment.

virgin biomass, points A, B, C, and D on the ERL are the only points on the SRA plot we need consider. This is because points A and D represent extremes of optimism and pessimism with regard to stock condition. From Table 4 we can see that solutions at point A are not consistent with the large decline in abundance that has been observed (Table 3). On the other hand, B_1 and R_1 at point D are too small to allow any solution to the SRA equations. Even these extreme scenarios are useful because they indicate with some confidence that $1\,000\,000\text{ t} \leq B_1 \leq 1\,500\,000\text{ t}$.

Of the remaining points B and C, the observed decline in abundance (Table 3) seems to favor point C. Therefore, we emphasize solutions at point C in our assessment.

The importance of the value of r in determining the sustainable yield of a population cannot be over emphasized. At point C, $r = 0$ and $r = 1$ represent extremes of optimism and

TABLE 4. Possible solutions to the SRA equations at points A, B, C, and D on the SRA plot (Fig. 2) for Pacific ocean perch in the Gulf of Alaska (B_{17} = estimated population biomass (tonnes) at the beginning of 1977).

	r	P	B_{17}
A. $B_1 = 1\,500\,000\ t$, $R_1 = 119\,021\ t$	0.0	0.606	909 598
	0.25	0.545	818 221
	0.50	0.494	741 249
	0.75	0.451	676 193
	1.00	0.414	621 007
B. $B_1 = 1\,250\,000\ t$, $R_1 = 99\,184\ t$	0.0	0.511	639 217
	0.25	0.433	540 703
	0.50	0.370	462 886
	0.75	0.321	400 992
	1.00	0.281	351 388
C. $B_1 = 1\,100\,000\ t$, $R_1 = 87\,282\ t$	0.0	0.416	457 283
	0.25	0.318	350 307
	0.50	0.247	272 016
	0.75	0.195	213 960
	1.00	0.155	170 268
D. $B_1 = 1\,000\,000\ t$, $R_1 = 79\,347\ t$	0.0	No solutions	
	0.25		
	0.50		
	0.75		
	1.00		

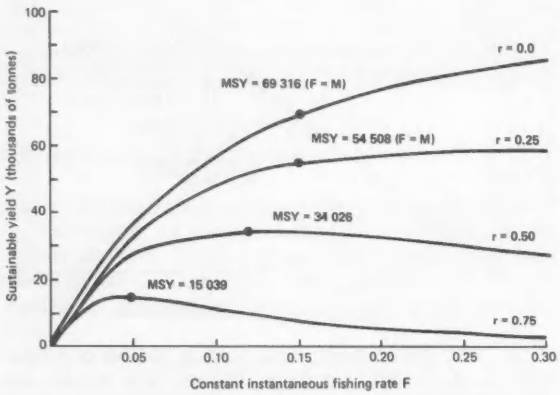
Sustainable yield Y (thousands of tonnes)
Constant instantaneous fishing rate F
MSY = 69 316 (F = M)
MSY = 54 508 (F = M)
MSY = 34 026
MSY = 15 039
r = 0.0
r = 0.25
r = 0.50
r = 0.75

FIG. 3. Sustainable yield curves for Pacific ocean perch in the Gulf of Alaska. Curves are calculated using equation 9 for point C on the SRA plot (Fig. 2).

pessimism with regard to recruitment. For $r = 0$, recruitment is constant and the population is resilient. For $r = 1$, recruitment is proportional to abundance and a population once reduced is incapable of rebuilding. By reexamining Fig. 1, the reader will get an intuitive feeling for the meaning of r. Briefly, if a stock has declined to $0.25B_1$, recruitment would be R_1 for $r = 0.0$, $0.71R_1$ for $r = 0.25$, $0.5R_1$ for $r = 0.5$, $0.35R_1$ for $r = 0.75$, and $0.25R_1$ for $r = 1.0$.

For point C on the SRA plot (Fig. 2), sustainable yield (calculated using equation 9) is plotted in Fig. 3 as a function of the instantaneous fishing mortality rate F, for various values of r. The results clearly demonstrate that MSY from a stock with even a moderate stock–recruitment relationship is severely limited. The corresponding equilibrium biomass curves (calculated using equation 8) corresponding to the yield curves in Fig. 3 are plotted in Fig. 4.

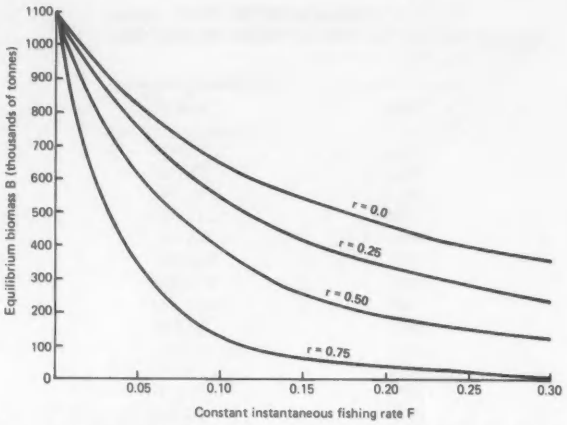

FIG. 4. Equilibrium biomass curves for Pacific ocean perch in the Gulf of Alaska. Curves are calculated using equation 8 for point C on the SRA plot (Fig. 2).

Considering the history of Pacific ocean perch in the Gulf of Alaska, the middle MSY value of 34 026 t when $r = 0.5$ does not seem overly conservative. However, our estimates of MSY using point C on the SRA plot are much lower than previous estimates (Table 5). Coincidentally, our estimate of virgin biomass (VB) using SRA is similar to an estimate based on cohort analysis (Table 5).

Archibald et al. (1983) recently completed an assessment of Pacific ocean perch in the Queen Charlotte Sound region using the model of Fournier and Archibald (1982). The results of their assessment compared with ours is shown in Table 6. Their results are similar to ours with r perhaps intermediate between $r = 0.5$ and $r = 0.75$. It should be understood that although Fournier and Archibald's model uses age data, it also includes a stock–recruitment relationship. From the point of view of MSY/VB, our assessment with $r = 0.5$ is more liberal than theirs.

Discussion

In examining stock assessments using a stock–recruitment relationship, it is important to remember the limitations of such an approach. The yield from a population will sometimes depend on the precise form of the stock–recruitment relationship, and this will usually be difficult if not impossible to resolve with gross population statistics. The assessments of Pacific ocean perch by Archibald et al. (1983) and ourselves are good cases in point. Archibald et al. (1983) estimated MSY to be 2.2% of VB, and we give a middle value of 3.1% of VB. We believe that these estimates may be overly conservative.

One reason MSY from these populations may be larger than calculated is that under near VB conditions, population dynamics may be approximated by a constant recruitment model. Perhaps only after the stock has been depressed to the neighborhood of one-half virgin biomass will we see the strong stock–recruitment relationship that dictates the low MSY/VB rates indicated by our assessments.

A useful method of assessment, then, is to examine the decline from VB for a constant recruitment model, when a

TABLE 5. Comparison of estimates using SRA at point C on the SRA plot (Fig. 2) with estimates from other authors for Pacific ocean perch in the Gulf of Alaska. All estimates are in tonnes.

	Source of estimates		
	SRA at point C	Shippen and Stark (1983)	Cohort analysis (Ito 1982)
Virgin biomass (VB)	1 100 000		987 607 (includes ages 9 yr and older)
Recruitment to VB (R_1)	87 282		
Maximum sustainable yield		125 000	
$r = 0.0$ (assuming $F = M$)	69 316 (high)		
$r = 0.25$ (assuming $F = M$)	54 508		
$r = 0.50$	34 026 (middle)		
$r = 0.75$	15 039 (low)		
$r = 1.0$	0		
Equilibrium yield for 1983		50 000	

TABLE 6. Comparison of our SRA assessment of Pacific ocean perch in the Gulf of Alaska with the assessment by Archibald et al. (1983) of Pacific ocean perch in Queen Charlotte Sound.

	SRA at point C		
	$r = 0.50$	$r = 0.75$	Archibald et al. (1983)
Virgin biomass (VB)	1 100 000 t	1 100 000 t	82 000 t
Maximum sustainable yield (MSY)	34 026 t	15 039 t	1 800 t
Equilibrium biomass at MSY (B at MSY)	323 548 t	331 855 t	37 000 t
MSY/VB	0.031	0.014	0.022
MSY/(B at MSY)	0.105	0.045	0.049
(B at MSY)/VB	0.294	0.302	0.451

constant annual quota is removed. For our SRA analysis, these values can be conveniently read from Fig. 3 and 4 (and Table 7). For example, under a constant recruitment model ($r = 0.0$) an annual quota of 34 026 t would reduce the VB (1 100 000 t) to 0.74 VB (about 815 000 t). This clarifies another important point: the same EY might be taken at more than one equilibrium level of stock biomass. In such cases, for the sake of population stability, it is best to take the yield from the higher biomass. For Pacific ocean perch in the Gulf of Alaska, we would recommend that the stock be rebuilt to 0.74 VB.

TABLE 7. Approximate proportion of virgin biomass remaining under a constant recruitment SRA model, when a constant annual quota is removed. SRA parameter estimates are at point C (Fig. 2), with $r = 0.0$.

Constant annual removal (quota) (t)	Proportion of virgin biomass remaining at equilibrium
69 316	0.49
54 508	0.61
34 026	0.74
15 039	0.88
0	1.00

Comparison of SRA with Other Stock Assessment Models

To place SRA in the context of current practice, we compare SRA with some well-known models used in stock assessment. We have not attempted here to review the extensive literature on stock assessment modeling.

A large number of commonly used stock assessment models can conveniently be classified as either Production Models (PM) or Virtual Population Analysis (VPA) models. The PM type models typified by Schaefer (1954), Pella and Tomlinson (1969), and Deriso (1980) have many characteristics in common. They all use catch and effort data, with catches recorded in weight, in an attempt to estimate parameters in a stock production curve.

The VPA type models typified by Murphy (1965), Pope (1972), and Fournier and Archibald (1982) also have many characteristics in common. They all attempt to solve the exponential form of the catch equations, with catches recorded in numbers at age, on an individual cohort basis.

The papers cited are only a miniscule sampling of a massive literature. Nevertheless they give some indication of the diversity to be found in each genre. Pella and Tomlinson (1969) generalized the parabolic PM, and applied a nonlinear least squares method of parameter estimation suitable for nonequilibrium conditions; Deriso (1980) introduced his delay-difference equation, which we have applied to SRA; Pope (1972) developed his approximation to VPA, and demonstrated convergence properties from his model; and Fournier and Archibald (1982) developed the most comprehensive likelihood approach to stock assessment that exists today. What is the relationship of SRA to these different methods?

Although SRA does not unequivocally fall into the PM group, it does appear to be much more similar to PM than VPA. Like PM, SRA models biomass rather than numbers, and provides estimates of sustainable yield. Unlike PM, SRA avoids the use of effort data and instead deals with a model saturated with parameters.

Because the Deriso delay-difference equation is implicitly age-structured, it shares similar assumptions with VPA models which are explicitly age-structured. Therefore, Deriso's (1980) PM and SRA, which are both based on the delay-difference equation, also share similar assumptions with VPA in regards to age-structure.

PM, VPA, and SRA each have their own weaknesses. PM generally assumes that fishing mortality is closely related to effort, and that there is a meaningful stock–production relationship. These assumptions have often proved to be inappropriate. VPA is dependent on knowing the catch at age in numbers. Age data are costly and technically difficult to obtain. Also, VPA has indeterminancies in its equations that are usually resolved by preliminary estimates of F and the linking of F values between cohorts, or by the application of effort data.

Perhaps the most important strength of SRA is that it does not require extensive age or effort data. Also, the model is formulated in such a way that all the parameters are intuitively easy to understand. Another important strength is that SRA can often use ancillary information in a straightforward manner. For example, what if P, or R, or B_i, or F_i were either known, or we wished to fix one of these values to determine its impact on an assessment? This can be done using SRA, but the impact of such constraints on most other forms of assessment would be difficult to evaluate. These are the properties of SRA that we hope will make the model useful.

The major weakness of SRA, which it shares with PM, is in the modeling of recruitment (or production) when recruitment is primarily driven by exogenous factors. Under these conditions, any attempt to model recruitment without ancillary information will fail. VPA avoids the variable recruitment problem and therefore has become a popular method of stock assessment. Here we have modeled exogenous variations in recruitment by using the proportional recruitment model (equation 6). With a proportional recruitment model, valid SRA plots can be made, but a resolution of solutions on these plots can only be made by estimating two additional variables among (B_1, R, P, F_1,..., F_n).

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Appendix A. Glossary of Variables Used in the Generalized Stock Reduction Analysis Model

Fundamental SRA Variables

i = the year index.

k = the age at recruitment to the fishable biomass, assumed to be known prior to SRA. It is used to estimate the Brody growth coefficient ρ (Appendix B), and to lag Cushing recruitment.

n = the number of years of catch data to be analyzed.

C_i = the catch in weight in year i; these are the numbers to be analyzed.

B_i = the fishable population biomass at the beginning of year i, including recruitment for that year.

R_i = the recruitment biomass to be added to the fishable stock at the beginning of year i.

R_0 = the recruitment biomass to be added to the fishable stock assuming a constant recruitment model.

R = the recruitment coefficient to be estimated using SRA, either R_1 or $R_{\max}$.

P = B_{n+1}/B_1, the ratio of final biomass to initial biomass.

Instantaneous Mortality Rates

M = the annual instantaneous natural mortality rate, assumed to be known prior to SRA.

F_i = the annual instantaneous fishing mortality rate for year i, to be estimated using SRA.

$Z_i = F_i + M$, the total instantaneous mortality rate for year i.

Parameters in the Deriso Delay-Difference Equation

s_i = $\exp(-Z_i)$, the survival in the fishable biomass in year i.

ρ = the Brody weight coefficient, estimated prior to SRA (see Appendix B).
W_ℓ = the average weight of a ℓ-yr-old fish; this is used for estimating ρ.
W_∞ = the average maximum attained weight for the oldest fish; this is used for estimating ρ.

Parameters in the Cushing Recruitment Model

r = the exponent in the Cushing recruitment model, assumed to be known prior to SRA.
R_1 = the Cushing recruitment coefficient to be estimated using SRA.

Parameters in the Proportional Recruitment Model

p_i = the proportion of R_{max} recruited to the population biomass at the beginning of year i, assumed to be known prior to SRA.
R_{max} = the recruitment coefficient for the proportional recruitment model to be estimated using SRA.

Parameters Related to Sustainable Yield

F = the fixed, long-term instantaneous fishing mortality rate for which B and Y are calculated.
s = the equilibrium survival rate in the fishable biomass.
U = the equilibrium exploitation rate.
B = the equilibrium population biomass under the fishing rate F.
Y = the equilibrium yield under the fishing rate F.

Appendix B. Method of Estimating the Brody Weight Coefficient

Let W_ℓ be the average weight of an ℓ-yr-old fish. For a fish recruited to the fishable biomass ($\ell \geq k$), the form of the Brody growth curve used by Deriso (1980) is

$$(10)\quad W_{k+j} = W_k(1 - \rho^{1+j})/(1 - \rho) \qquad j \geq 0, 0 \leq \rho < 1.$$

A simpler form of this equation is

$$W_{k+j} = W_k(1 + \rho + \rho^2 + \ldots + \rho^j).$$

Another useful form of this equation is

$$W_{k+j} = W_\infty(1 - \rho^{1+j})$$

where

$$(11)\quad W_\infty = W_k/(1 - \rho)$$

is the maximum attainable average weight.

Clearly, from this last form of the Brody growth curve, our estimate of ρ is a function of the age at recruitment k. If k is large (i.e. recruitment to the fishable biomass is late), then $W_k \approx W_\infty$ and $\rho \approx 0$ in the fishable biomass. This behavior is consistent with our use of the model in SRA.

The first step in estimating ρ is to estimate the age at recruitment k. For most stocks of fish, recruitment is not knife-edged, so only an approximation can be made. Using the argument given in the text that if ρ is underestimated a compensation will be made in recruitment, we consider it better to overestimate rather than underestimate k. Under this assumption, an acceptable estimate of k may be the modal age of the catch. However, the problem is one that may deserve further research.

Once an estimate of k is available, the problem becomes one of statistical curve fitting. Nonlinear least squares can be used to estimate ρ from equation 10,

$$(12)\quad Y_j = W_{k+j}/W_k \approx (1 - \rho^{1+j})/(1 - \rho).$$

Under unusual circumstances when only the average weight at recruitment and maximum attainable average weight are known, it may be possible to estimate ρ using equation 11,

$$(13)\quad \hat{\rho} = 1 - W_k/W_\infty.$$

Lethal and Sublethal Toxicity of Drilling Fluids to Larvae of the American Lobster, *Homarus americanus*[1]

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Derby, J. G. S., and J. M. Capuzzo. 1984. Lethal and sublethal toxicity of drilling fluids to larvae of the American lobster, *Homarus americanus*. Can. J. Fish. Aquat. Sci. 41: 1334–1340.

The lethal and sublethal effects of five used, whole drilling fluids on the larval stages of the American lobster (*Homarus americanus*) were assessed in laboratory experiments using a continuous-flow bioassay. Although the five tested drilling fluids varied markedly in their toxicity, some were highly toxic, with LC50 values as low as 74 mg/L. Sublethal exposures to drilling fluids at concentrations as low as 10–50 mg/L resulted in reduced respiration rates, reduced O:N ratios, and increased protein:lipid ratios, demonstrating a change in energetics of the larval lobsters. Growth and development of the larvae were seriously impaired by exposure to three of the five drilling fluids at concentrations of 50 and 100 mg/L. The feeding rates were also significantly reduced after a 24-h exposure to 50 mg/L drilling fluid. Exposure of larvae to barite (a major component of drilling fluids) and to a field-collected, fine-grained sediment did not result in deleterious effects. We suggest that the chemical components and not the physical properties of the drilling fluids are primarily responsible for detrimental effects. From results of the chemical analyses of the tested drilling fluids, we consider that the adverse effects of these drilling fluids cannot be attributed to any one group of chemicals. For example, diesel oil, a known toxicant, was present in the more toxic drilling fluids; however, there was no direct correlation between the toxicity of a drilling fluid and diesel oil concentration. Phenolic compounds, various metals, and other components probably also contributed to the toxicity of these drilling fluids.

À l'aide de dosages à débit continu, on a évalué les effets létaux et sublétaux de cinq fluides de forage entiers usés sur les stades larvaires du homard d'Amérique, *Homarus americanus*, en laboratoire. Quoique la toxicité des cinq fluides de forage testés variait grandement, certains étaient très toxiques : la valeur de la DL50 pouvait être aussi basse que 74 mg/L. Des expositions sublétales à des concentrations aussi faibles que 10 à 50 mg/L entraînaient des taux respiratoires réduits, des rapports O:N abaissés et des rapports protéine:lipide accrus, manifestations d'un changement de l'énergétique des homards larvaires. La croissance et le développement des larves étaient grandement diminués par l'exposition à trois des cinq fluides de forage à des concentrations de 50 et 100 mg/L. De plus, les taux d'alimentation étaient nettement réduits après une exposition de 24 h à une concentration de 50 mg/L. L'exposition des larves à la barytine (une composante importante des fluides de forage) et à un fin sédiment recueilli sur le terrain n'a pas occasionné d'effets néfastes, ce qui porte à croire que ce sont les composantes chimiques, et non les propriétés physiques des fluides de forage, qui sont essentiellement responsables des conséquences nuisibles. Les résultats d'analyses chimiques des fluides de forage testés indiquent que leur incidence négative ne peut pas être attribuée à un groupe particulier de produits chimiques. Par exemple, l'huile diesel, dont la toxicité est reconnue, était présente dans les fluides de forage les plus toxiques ; toutefois, il n'y avait aucune corrélation directe entre la toxicité du fluide de forage et la concentration d'huile diesel. Des composés phénoliques, divers métaux et d'autres composantes contribuaient probablement à la toxicité des fluides de forage.

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Oil and gas drilling operations in offshore regions, including the Northwest Atlantic, pose many unanswered questions concerning the impact of pollutant discharges on marine ecosystems. Our study explores the effects of drilling fluids, a major effluent from ocean drilling activities, on aspects of the physiology of an economically important cold-water invertebrate, the American lobster (*Homarus americanus*).

Drilling fluids (also called drilling muds) are complex mixtures of clays and chemical additives that are used in drilling operations to equalize hydrostatic pressure, remove drilled cuttings, and lubricate the drill bit. Drilling fluids along with drill bit cuttings are continually discharged to the surrounding

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TABLE 1. Chemical and physical characteristics of the J-series and Mobile Bay drilling fluids,[a] barite (Bar), and Georges Bank sediment (GB).

Drilling fluid	Total metals						Diesel oil (%)	Total resolved aliphatics (μg/L)	Total resolved aromatics (μg/L)	Phenols (mg/L)	Clay (%)	Silt (%)	Sand[b] (%)
	Fe (%)	Al (%)	Ba (%)	Pb (mg/L)	Cr (mg/L)	Zn (mg/L)							
J-4	3.2	8.0	4.1	40.2	96.4	225.0	0	17 000	27 300	<0.25	55.7	42.3	2.0
J-5	2.6	6.5	9.3	41.7	201.0	271.0	2	1 320 000	380 000	0.45	42.6	55.3	2.3
J-6	2.3	5.9	10.6	41.3	160.0	250.0	3	—	555 000	<0.25	55.0	43.1	1.9
J-7	1.1	2.5	11.7	18.5	128.0	175.0	4	1 270 000	376 000	0.33	58.1	39.9	1.9
MB-5-29	2.7	6.1	8.2	37.8	1810.0	98.6	0.27[c]	—	—	1.3	36.1	64.5	1.0
Bar	—	—	—	—	—	—	—	—	—	—	30.3	64.8	4.9
GB	—	—	—	—	—	—	—	—	—	—	8.9	50.2	41.0

[a]Analyses performed at Science Applications, Inc., La Jolla, CA; New England Aquarium, Boston, MA; University of West Florida, Pensacola, FL; Energy Resources Co., Inc., Cambridge, MA; U.S. Geological Survey, Woods Hole, MA. Dashes indicate data not available.
[b]Grain size determined using Coulter Counter.
[c]No. 2 fuel oil.

waters during the drilling operation and are discharged in bulk at the completion of a well or during a major change in the drilling fluid program. Previous studies of drilling fluid effects on juvenile and adult crustaceans have demonstrated changes in burrowing behavior, food detection capabilities, molting frequency (Atema et al. 1982), respiration and growth rates (Carr et al 1980), and interference of chemosensory processes (Derby and Atema 1981); drilling fluid concentrations responsible for inducing sublethal effects, however, varied widely (7.7–35 000 mg/L). Little is known of the susceptibility of larval stages to drilling fluids.

The objective of our study was to determine if alterations in the energetics of larval development in the American lobster resulted from exposure to used, whole drilling fluids. The toxicities of several types of drilling fluids were compared by measuring survival, metabolism, energy utilization, and biochemical and mineral compositions. These studies were conducted in a continuous-flow bioassay, the advantages of which are described in Derby and Capuzzo (1984). The effects of a major drilling fluid component, barite, and a fine-grained sediment collected in the field were also established in an effort to determine the physical versus the chemical effects of drilling fluid.

Materials and Methods

Drilling Fluids

The used, whole drilling fluids tested in these experiments were supplied by the U.S. Environmental Protection Agency, Gulf Breeze, FL, and are characterized in Table 1. The J-series fluids (J-4 through J-7) were collected in July 1980 from a land-based well in Jay, FL, at drilling depths of 3736, 4084, 4266,and 4438 m, respectively. The Mobile Bay drilling fluid collected on May 29, 1979 (MB-5-29), had been used in the Gulf of Mexico at drilling depths of about 4420 m. All drilling fluids were stored at 4°C. The drilling fluids ranged in water content from 79 to 88%, density from 1.09 to 1.11 kg/L, and pH from 11.0 to 11.9.

Bioassay System and Techniques

Egg-bearing lobsters were obtained from Vineyard Sound, Cape Cod, MA, by local fishermen and were placed in our laboratory holding tanks at ambient seawater temperatures and salinity (18–22°C, 30–31‰). After hatching, stage I larvae were transferred to fiberglass plankton-kreisels, described by Hughes et al. (1974). Tests conducted with stage I larvae began within 24 h of hatching. The later larval stages used in the bioassays were reared in the kreisels on a diet of frozen adult brine shrimp, after being thawed and rinsed.

Bioassays with MB-5-29 were conducted during the summer of 1979 and the J-series drilling fluids were tested in the summers of 1980 and 1981. Larval lobsters were exposed to used, whole drilling fluids in a modification of the continuous-flow bioassay system described by Capuzzo et al. (1976). In this system, seawater–pollutant mixtures were supplied by peristaltic pumping to thirty-two 500-mL fleakers® (Corning Glass), which served as assay chambers. Modifications of the system were made to allow for a uniform distribution of a seawater–drilling fluid mixture in the head-tanks and in the individual test chambers. The head-tanks (eight 13-L carboys) were supplied with magnetic stir bars and placed on electric magnetic stirrers. Each assay chamber held one stir bar, and rested on a water-propelled magnetic stirrer. A false bottom of 1-mm Nitex® screening secured with clear, silicone sealant to the chamber floor prevented contact between the test organisms and the rotating stir bar. The test chambers were held in a water bath to maintain constant temperature during the assay period. Replacement time in each test chamber, calculated according to Sprague (1969), was estimated to be 99% replacement of water in approximately 7.5 h. The experimental temperatures and dissolved oxygen concentrations within the test chambers ranged from 18 to 24°C and 7.3 to 8.1 mg/L, respectively, over the summer season.

Larval lobsters were exposed for 96 h to concentrations of drilling fluids ranging from 1 to 500 mg/L. Lobsters exposed to filtered seawater and suspensions of barite (Bar) (Milbar brand) and a fine-grained sediment collected from the Georges Bank "mud patch" area (GB) served as control experiments. Five larvae were added to each assay chamber and were maintained on a diet of live *Artemia* nauplii (Brazilian strain, Aquafauna, Inc.) at a density of 100–200 nauplii per larva. Larvae were monitored for survival, respiration rates, ammonia excretion rates, and O:N ratios at selected times during the 96-h exposure period. Moribund larvae were examined microscopically; lack of heartbeat was used as the criterion of death. Control and exposed larvae were rinsed in distilled water and freeze-dried

after the 96-h exposure period. The dry weights of individual larvae were measured using a Cahn Electrobalance®.

An additional control experiment was conducted with stage I larval lobsters fed with *Artemia* nauplii that had been previously exposed to drilling fluid. Larvae were held in the continuous-flow bioassay system described. The *Artemia* hatching and exposure system consisted of a series of 500-mL glass separatory funnels, fitted with glass rods for gentle aeration. Newly hatched *Artemia* nauplii were exposed to concentrations of J-5 drilling fluid ranging from 50 to 500 mg/L for 12 h and were then rinsed with clean seawater. Five lobster larvae were placed in the assay chambers and were supplied with 200 exposed nauplii per larva daily for 96 h. Respiration rates, ammonia excretion rates, and O:N ratios were measured at 96 h and were compared with control organisms maintained under identical conditions. Comparisons were also made on survival, individual dry weights, and molt stage of control and experimental larvae.

In another set of experiments, food consumption of stage I larvae exposed to drilling fluid for 24 h was measured by adding a known quantity of *Artemia* nauplii and counting those remaining after 1 h. For measurement of feeding rates, individual larvae were transferred to 100-mL glass vials with filtered seawater, GB, or seawater–drilling fluid mixtures. Data from these experiments were converted from the number of *Artemia* consumed to joules using average dry weight and energy content for *Artemia* nauplii. Growth rates of larvae were also converted to hourly values using the energy equivalents of dry weights of stage I larvae given by Logan and Epifanio (1978) converted to joules.

Respiration rates of seven lobsters per treatment in each experiment were measured using a Gilson differential respirometer according to the techniques described by Capuzzo and Lancaster (1979); ammonia excretion rates were measured according to the method of Solorzano (1969). The O:N ratios (atomic ratio of oxygen consumed to ammonia-N excreted) were determined for lobsters exposed to control seawater and to drilling fluid (Capuzzo and Lancaster 1979).

The relative percentages of protein, carbohydrate, lipid, and ash were determined for 96-h exposed and control lobsters. Freeze-dried larval lobsters were ashed at 475°C for 8 h in a muffle furnace. Protein extraction was accomplished with the modification of methods previously described by Holland and Gabbott (1971). The Bio Rad protein dye binding assay was used to quantify protein concentrations (van Kley and Hale 1977). Lipids were extracted by the combined methods of Bligh and Dyer (1959) and Folch et al. (1957) and were gravimetrically quantified. The carbohydrate assay employed was described by Raymont et al. (1964).

Elemental analysis for barium and calcium were carried out on 5.0–10.0 mg pooled samples of dried, stage I larvae from both exposed and control groups, by means of instrumental neutron activation analysis. Irradiations were carried out at the Rhode Island Nuclear Science Center according to the techniques described by Spencer et al. (1978).

Means and groups of means were compared by analysis of variance. Median lethal concentrations (LC50) and confidence limits at 96 h were estimated by the BMD-035 revised program of probit analysis (Dixon 1981). The program allowed for log transformation of exposure concentrations and corrected for mortality in the controls (Finney 1971).

TABLE 2. Mean dry weights of stage I larvae exposed to control seawater (CSW), Georges Bank sediment (GB), and barite (Bar) at 24 and 96 h.

Group	Dry weight (±1 SE), mg (*n*)	
	24 h	96 h
CSW	1.00±0.05 (13)	1.45±0.05 (32)
GB, 500 mg/L	1.07±0.02 (14)	1.58±0.04 (30)
Bar, 500 mg/L	1.03±0.03 (14)	1.59±0.05 (33)

Results

Control Experiments

The percent survival at 96 h for larvae exposed to control seawater (CSW) (73 ± 8) was not significantly different from larvae exposed to GB at 500 mg/L (63 ± 3) or to Bar at 500 mg/L (60 ± 7). The effects of CSW, GB, and Bar were not significantly different for all five sublethal characteristics measured (dry weight data in Table 2). Thus, any observed toxic effects of drilling fluid are primarily due to chemical and not physical characteristics of drilling fluid mixtures. An additional control experiment examined the effect of ingestion by lobster larvae of *Artemia* nauplii that were exposed to drilling fluid. The consumption of these exposed *Artemia* by the lobsters had no effect on their survival, growth, respiration, or ammonia excretion rates (survival and dry weight data in Table 3) with the exception of lobster larvae fed *Artemia* nauplii exposed to 500 mg/L of J-5. This demonstrates that any observed effects of drilling fluid on lobsters are primarily due to direct exposure to drilling fluids rather than indirect exposure through ingestion of previously contaminated food or bioaccumulation of drilling fluid components through the food chain.

TABLE 3. Percent survival (mean of four replicates) and mean dry weights at 96 h of stage I larvae fed *Artemia* nauplii in uncontaminated seawater (CSW), Georges Bank sediment (GB), and J-5 drilling fluid.

Artemia group	% survival (±1 SE)	Dry weight (±1 SE), mg
CSW	70.0±12.9	1.65±0.09
GB, 50 mg/L	65.0±5.0	1.81±0.05
J-5, 50 mg/L	75.0±5.0	1.76±0.04
J-5, 100 mg/L	80.0±8.2	1.66±0.05
J-5, 250 mg/L	65.0±12.6	1.60±0.07
J-5, 500 mg/L	60.0±0.0	1.42±0.04[a]

[a] $P < 0.025$.

TABLE 4. Four-day median lethal concentration (LC50) (±95% confidence limits) for drilling fluid exposed lobster larvae.

Drilling fluid	Larval stage of lobster	96-h LC50 (mg/L) (95% CL)
MB-5-29	I	190 (134–268)
MB-5-29	III	117 (52–261)
J-4	I	>500
J-4	IV	>500
J-5	I	74 (52–105)
J-6	I	213 (80–564)
J-7	I	88 (62–124)

TABLE 5. Mean dry weights (±1 SE) (mg) for stage I larvae exposed to control seawater (CSW) and to drilling fluid for 96 h.

	CSW	Type of drilling fluid	Concn. of drilling fluid, mg/L		
			10	50	100
	1.27 (0.03)	J-4	1.22[a]	1.27[a]	1.18[a]
% molt to stage II	100		100	100	100
	1.62 (0.05)	J-5	1.35 (0.10)	1.13 (0.04)[b]	0.88 (0.02)[b]
% molt to stage II	86		75	43	0
	1.57 (0.08)	J-6	1.61 (0.07)	1.43 (0.04)	1.19 (0.10)[c]
% molt to stage II	100		100	95	50
	1.46 (0.06)	J-7	1.26 (0.04)	1.37 (0.05)	1.16 (0.03)[d]
% molt to stage II			94	92	75
	0.96	MB-5-29[a]	0.98	0.74	0.75

[a]Average dry weight per larva from pooled samples.
[b]$P < 0.001$.
[c]$P < 0.025$.
[d]$P < 0.005$.

Mortality

Four-day LC50 values demonstrate a wide variability in the toxicity of the five drilling fluids tested (Table 4). Based on the mortality data for stage I larvae, the order of toxicity from the greatest to the least is J-5, J-7, MB-5-29, J-6, and J-4. The average percent mortality value for the CSW group in all experiments was 33%. Mortalities among communally held larval lobsters are high as compared with individually held larvae (Sastry and Zeitlin-Hale 1977) and can be attributed to cannibalism or group interactions that lead to cannibalism. The number of larvae per test chamber used in our experiments may have introduced a higher degree of stress than if we had used only one larva per chamber. Capuzzo and Lancaster (1982), however, observed no differences in physiological responses of larval lobsters exposed to petroleum hydrocarbons individually or communally. Thus, it would not appear that communal exposure of lobster larvae to drilling fluids alters the validity of the observed findings.

Physiological Effects

In comparison with control values, significant decreases in larval growth rates and delays in development times (as indicated by time between molts) resulted from both 24- and 96-h exposures to drilling fluids (Table 5). Decreases in mean dry weights of stage I larvae were found after a 24-h exposure to J-6 at 500 mg/L ($P < 0.005$) and J-7 at 500 mg/L ($P < 0.01$). After a 96-h exposure, J-5 was the most toxic drilling fluid tested in terms of these two factors, followed by J-6 and J-7. The J-4 drilling fluid at all concetrations tested did not alter growth rates or development times of stage I or stage IV larvae.

Respiration rates calculated on a weight-specific basis (expressed as microlitres O_2 consumed per hour per milligram dry weight) were not statistically different for most of the groups of control and exposed stage I larvae (Fig. 1). After 24 h of exposure, only larvae exposed to MB-5-29 at 10 mg/L and J-5 at 100 mg/L had significantly reduced weight-specific respiration values ($P < 0.05$ and $P < 0.005$, respectively). At 96 h, decreased respiration rates ($P < 0.05$) continued to be observed among lobster larvae exposed to 10 mg/L MB-5-29. However, at 96 h, no significant differences were found for stage I larvae exposed to any of the J-series drilling fluids, although the mean weight-specific respiration value was reduced for each as compared with the controls (Fig. 1). Respiration rates for stage IV larvae exposed to J-4 were determined at 24-, 48-, 72-, and 96-h exposure periods. There were no significant differences in respiration rates between control larvae and larvae exposed to 100 and 500 mg/L.

Ammonia excretion rates of stage I larval lobsters were significantly increased during the 96-h exposure to two of the drilling fluids tested, J-5 and J-7. Values for the atomic ratio of oxygen consumed and ammonia-N excreted were significantly lower among stage I larvae exposed to J-5, J-6, and J-7 for 96 h (Fig. 2). Exposure to J-4 drilling fluid resulted in no differences in ammonia excretion rates or O:N ratios between exposed and control larvae for both stages I and IV, with the exception of a significant increase in the 72-h ammonia excretion rates of stage IV larvae exposed to 500 mg/L J-4.

Stage I larvae exposed to a sublethal concentration of the most toxic drilling fluid tested, J-5, had significantly reduced feeding rates. The feeding rates after a 24-h period were 16 (±3) (mean ± 1 SE) *Artemia* nauplii larva per hour for larvae exposed to 50 mg/L J-5, 27 (±3) for the CSW group, and 33 (±3) for the GB group. A statistical comparison demonstrated a highly significant difference ($P < 0.001$) between the controls and the drilling fluid group. The energy utilized for growth (G) was also considerably lower for the drilling fluid exposed larvae in comparison with the controls, resulting in reduced growth efficiencies (K_1) (Table 6).

Biochemical Effects

Biochemical compositions of stage I and stage IV larval lobsters were altered after exposures to drilling fluid (Table 7). Significant increases in protein content ($P < 0.01$) and in protein:lipid ratios ($P < 0.05$) were found in stage I larvae exposed to 50 mg/L J-5. Biochemical analysis of stage IV larvae exposed to 100 mg/L J-4 drilling fluid showed a significant increase ($P < 0.05$) in lipid content. The lobster larvae exposed to drilling fluid had the highest measured calcium concentrations, as compared with controls. The tissues of larvae exposed to 250 and 500 mg/L drilling fluid (J-4)

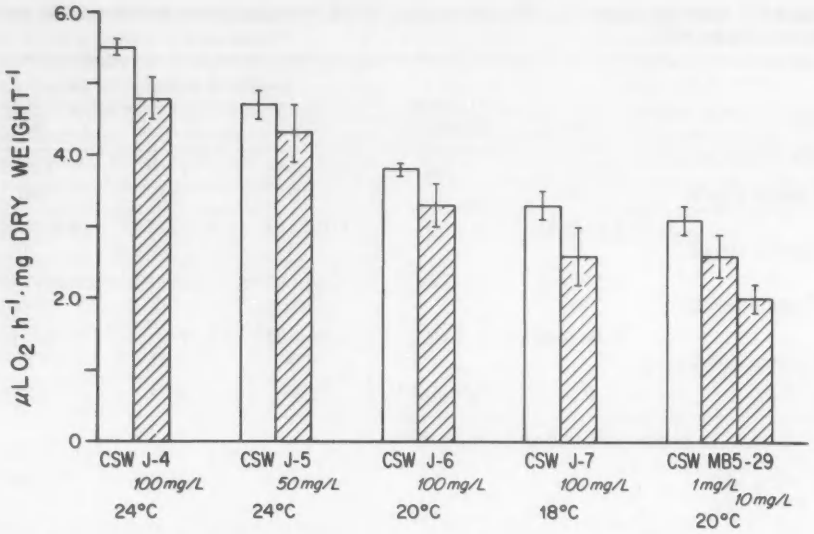

FIG. 1. Effects of drilling fluids on the respiration rates of stage I larvae (mean ± 1 SE at 96 h, ambient seawater assay temperature). CSW, control seawater.

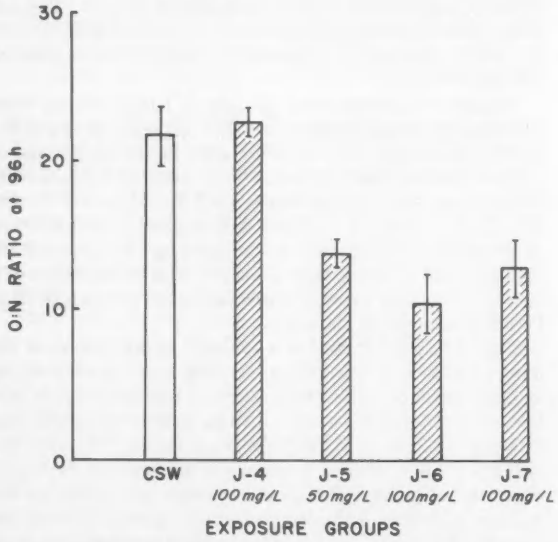

FIG. 2. Effects of the J-series drilling fluids on the O:N ratios of stage I larvae (mean ± 1 SE at 96 h).

contained 43.8 and 33.0 μg Ca/mg, respectively. The control values were 29.2 μg Ca/mg for larvae exposed to CSW and 24.4 μg Ca/mg for larvae exposed to 500 mg/L Bar. A barium concentration of 1.43 μg/mg dried tissue was found for the larvae exposed to Bar; barium was not detected in the other groups.

Discussion

The diverse lethal and sublethal toxicities of the different drilling fluids tested in this study demonstrate the importance of testing a range of drilling fluids in terms of chemical complexity, in order to adequately evaluate the potential impacts of offshore discharge. The LC50 values for the most toxic drilling fluids tested (74, 88, and 117 mg/L) are considerably lower than the values reported for other marine crustaceans and with most other drilling fluids (Bookhout et al. 1984; and reviewed in Derby and Capuzzo 1984).

Our study has concluded that the chemical and not the physical features of drilling fluids are primarily responsible for the harmful effects of drilling fluids. Exposure to both barite and Georges Bank sediment caused no sublethal or lethal effects on the lobster larvae, even at very high concentrations (500 mg/L). Exposure to most of the drilling fluids, however, resulted in changes in survival and energetics at concentrations generally ranging from 50 to 100 mg/L. It should be noted, however, that experiments by Conklin et al. (1980) revealed histopathological evidence of perturbations in the midgut epithelium in another crustacean, *Palaemonetes pugio*, exposed to concentrations of barite comparable with those in this study. Possibly, the chemically based toxicity demonstrated with these drilling fluids may act in combination with such perturbations as those resulting from the physical properties of the clays in drilling fluid.

Whole drilling fluids were tested in order to simulate as closely as possible the chemicals that lobsters will be exposed to

TABLE 6. Growth efficiencies (K_1) for control and drilling fluid exposed stage I larvae at 24 h.

Group	C (joules)[a]	G[b]	K_1[c]
CSW	0.75	0.15	20.0
GB-50, mg/L	0.90	0.13	14.5
J-5-50, mg/L	0.42	0.02	4.8

[a]Based on x values of 16 determinations of number of *Artemia* nauplii consumed per hour converted to joules of *Artemia* per weight.
[b]Daily increases in dry weight converted to joules.
[c]$K_1 = G/C \times 100$.

TABLE 7. Biochemical analyses of lobster larvae exposed to control seawater (CWS) and drilling fluid (mean ± 1 SE) on an ash-free dry weight basis with a sample size of 5 with 3 replicates per sample.

Stage	Exposure group	Component				Protein: lipid ratio
		% ash	% protein	% carbohydrate	% lipid	
I	CSW	21.5±1.7	57.8±1.4	1.4±0.2	11.6±0.9	5.0
	GB, 50 mg/L	23.6±1.6	60.2±0.7	1.2±0.2	11.9±0.3	5.1
	J-5, 50 mg/L	22.8±2.2	63.7±1.2[a]	1.2±0.1	11.4±0.3	5.6[b]
IV	CSW	31.2±0.9	37.4±1.3	3.5±0.2	17.1±0.2	2.2
	J-4, 100 mg/L	31.6±0.2	35.6±1.4	3.2±0.1	18.3±0.2[b]	1.9

[a] $P < 0.01$.
[b] $P < 0.05$.

with ocean discharges. There are two factors that make it difficult to identify which classes of constituents are primarily responsible for the toxicity of each drilling fluid used in this study: (1) the chemical compositions of the drilling fluids are incompletely known and (2) there is the possibility of synergistic interactions among the many constituents. Nevertheless, certain chemicals known to be toxic to some marine crustaceans are present in the drilling fluids studied here, including petroleum products, metals, and phenols (Capuzzo and Lancaster 1981; Brannon and Rao 1979; Caldwell et al. 1977; Cantelmo et al. 1978; Wells and Sprague 1976).

Important physiological functions including growth rates, development times, respiration rates, and feeding rates were detrimentally altered with exposure to sublethal concentrations ranging from 10 to 100 mg/L. Decreased food consumption was an important contributing factor to the reduced growth rates and growth efficiencies observed among exposed lobsters. Lower food consumption rates may be attributed to numerous variables, such as weakened condition of larvae exposed to drilling fluids (Atema et al. 1982), interference of chemosensory processes (Derby and Atema 1981), or changes in the palatibility of the food source (Sutterlin 1974).

Disruption in energy flow, manifested specifically as impairment of growth and development, may be the most serious consequence of drilling fluid exposure for larval lobsters. The reduced respiration rates of lobster larvae exposed to some drilling fluids reflect an interference of drilling fluid components with metabolic processes that could result in inefficient utilization of food and subsequent reductions in growth. Change in energy storage and utilization patterns (as reflected by changes in O:N ratios and biochemical composition) provides a further indication that alterations in energy cycles may result from drilling fluid exposure. The reduction in O:N ratios indicates an increased dependence on protein catabolism for energy production. Increased protein catabolism, protein content, and protein:lipid ratios of exposed larvae suggest a failure to store high energy lipid substrates, reducing the energy reserves available for growth and development.

The drilling fluids with a diesel component and with relatively high chromium and phenol levels were the most toxic drilling fluids tested. The drilling fluid J-4, having the lowest barium, chromium, zinc, phenol, and diesel content, was the least toxic, as demonstrated by the LC50 values and all of the sublethal measurements. The most toxic J-drilling fluid, J-5, had a lower diesel content than J-6 or J-7; however, chromium and phenol levels were higher in this drilling fluid. MB-5-29 also had a relatively low diesel content and considerably higher chromium and phenol concentrations as compared with the J-series. Larvae exposed to MB-5-29 had an LC50 value closely comparable with J-6 exposed larvae; sublethal effects resulting from MB-5-29 exposures were seen at concentrations as low as 10 mg/L. Although a direct correlation between percent diesel and relative toxicity was not established, the presence of a diesel oil component was an important contributing factor to drilling fluid toxicity. Other studies have also indicated that those drilling fluids containing a diesel oil component are more toxic than those that do not (Derby and Capuzzo 1984; T. P. Gilbert et al., New England Aquarium, Boston, MA, unpubl. rep. submitted to USEPA; Gerber et al. 1980). Such a finding is supported by a vast literature on the highly toxic effects of petroleum hydrocarbons to crustaceans (e.g. Capuzzo and Lancaster 1981; Johns and Pechenik 1980; Atema et al. 1979; Edwards 1978; Wells and Sprague 1976). Certain sublethal effects of drilling fluids including the interference of chemosensory processes (Derby and Atema 1981) and the shifts in the biochemical compostion of energy reserves (present study) have also been observed with exposure to petroleum hydrocarbons (Capuzzo and Lancaster 1981; Atema et al. 1979).

The likelihood that lobster larvae as a component of the plankton will encounter concentrations of drilling fluids comparable with those used in these experiments will be dependent on dispersion rates and distribution patterns of drilling fluid discharges at specific drilling sites. Specific location of the drilling site in terms of local atmosphere and hydrographic conditions may result in highly variable dispersion patterns. Studies have been conducted on the distribution and concentration of the solid fraction but not the soluble fraction of discharged drilling fluids in the vicinity of drill sites (Ayers et al. 1980; Gettleson and Laird 1980; Dames and Moore, Inc., Los Angeles, CA, unpubl. rep for Shell Oil Company). Concentrations of suspended solids and barium were measured in these studies in an effort to trace the drilling fluid plumes, and results indicate that solid components of drilling fluids can be dispersed in detectable quantities at least 1000 m from the source. The Bureau of Land Management (1979) indicated that plumes of drilling fluids are often visible 4–5 km downstream from the release site. The results of these field monitoring studies, however, provide no answers to questions concerning the fate of the chemical fractions of drilling fluids in the water column. Our study has shown that these fractions are of greatest concern in terms of causing detrimental effects to the early life stages of a marine organism. Further studies are needed to determine

whether these chemical constituents remain associated with particulates in the discharge plume or solubilize and become more rapidly diluted.

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Selection of External Tags for Marking Atlantic Herring (*Clupea harengus harengus*)

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Of seven external tags (carlin, disc and disc, disc and dangler, anchor, short anchor, dart, streamer) applied to Atlantic herring (*Clupea harengus harengus*), the anchor tag is the best to use when tagging rate, recovery rate, and persistence of tagged fish in the population are considered. A high recovery rate for carlin tags was more than compensated by the higher application rate exhibited by anchor and dart tags. Anchor tagged fish had a lower sustained mortality and reduced time in setting up the tags for application compared to dart tags. Tagged herring suffered higher short and apparent long-term mortalities than the unmarked population regardless of which external tag type was applied. Long-term tag loss may have been due to tag induced mortality and/or tag shedding. Consequently, studies requiring data beyond 2 yr after release would need another type of tag. Initial mortalities of herring increased with holding and handling time for all tags tested. This result confirms that herring should be tagged immediately after capture to reduce initial mortalities.

Des sept types d'étiquettes externes (carlin, disques doubles, disque et spaghetti, à ancrage, courte à ancrage, à dard, ruban) attachées au hareng atlantique (*Clupea harengus harengus*), l'étiquette à ancrage est la meilleure pour ce qui est du taux d'étiquetage, du taux de récupération et de la permanence du poisson étiqueté dans la population. Le taux élevé de récupération des étiquettes carlin a été plus que neutralisé par le taux supérieur de fixation des étiquettes à ancrage et à dard. Il a fallu moins de temps pour préparer les étiquettes à ancrage avant la fixation et les poissons ainsi étiquetés ont accusé une plus faible mortalité que ceux portant des étiquettes à dard. Quel que soit le type d'étiquette, les harengs étiquetés ont subi une plus haute mortalité à court terme et une plus haute mortalité apparente à long terme que la population non marquée. La perte d'étiquettes à long terme peut avoir été causée par la mortalité due à l'étiquette ou par la perte de celle-ci. Par conséquent, les études qui requièrent des données pour plus de deux ans après la mise en liberté nécessiteront un autre genre d'étiquette. La mortalité initiale du hareng a augmenté avec le temps de stabulation et de manutention pour tous les types d'étiquettes étudiés. Ce résultat corrobore la théorie selon laquelle on devrait étiqueter le hareng immédiatement après la capture afin de réduire la mortalité initiale.

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Tagging experiments remain one of the most widely used methods to gather information on fish population parameters. With proper care in experimental design a substantial quantity of data may be accrued with relatively small effort. In fisheries, tagging studies have been employed to estimate population size (Jones 1976; Bowen and Sergeant 1983), to validate ages (Brown et al. 1983), to determine migration patterns (Haegele et al. 1982; Winters and Beckett 1978), to estimate mortality rates (Seber 1965), and to separate fish stocks (Haegele et al. 1982; Pristas et al. 1976). However, a failure to address assumptions in the data analysis and model selection phase may prove critical in interpreting the results of tagging experiments (see Ricker 1975).

One of the fundamental problems in mark–recapture studies is the choice of tag to be applied (Jones 1976). Several criteria such as the method of discovery and reporting, the effects of differential mortality between tagged and untagged fish, the shedding of tags with time, and ease of application have to be considered by the researcher prior to initiating a long-term tagging program.

In the Newfoundland region, studies on migration patterns and stock discrimination of Atlantic herring (*Clupea harengus harengus*) have been conducted since the late 1960's. Information from tagging experiments has proven instrumental in elucidating stock interrelationships and migration patterns that are particularly germane to managing herring stock units (Winters and Beckett 1978; Wheeler and Winters 1984a). The earlier herring tagging experiments utilized internal metallic tags whose application and recovery characteristics have been described elsewhere (Winters 1977). The more recent herring tagging experiments in the northwest Atlantic mainly have involved a variety of external tags whose relative merits have not been evaluated insofar as optimum tag type is concerned. This paper presents the results of such comparisons based upon recovery data from a series of systematic herring tagging experiments conducted in the Newfoundland area during the 1970's.

Materials and Methods

Live herring were obtained from a variety of commercial fishing gear such as traps, bar seines, or purse seines and held in

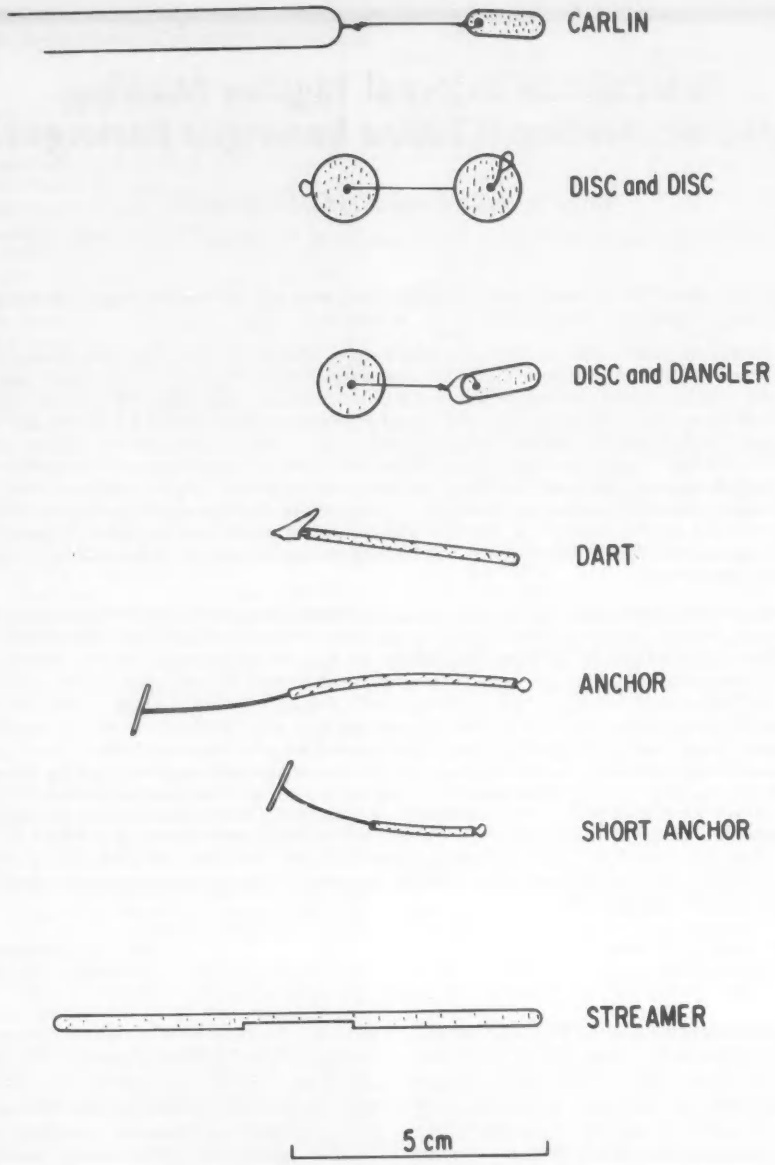

FIG. 1. Seven external tags compared in the study.

holding pounds (Winters and Beckett 1978) until tagged. The tagging procedure itself is more fully described in Wheeler and Winters (1984b).

Seven different tag types were applied (Fig. 1). Recovery data were evaluated according to the criteria of application rate, retention time, tag-induced mortality, and thus, directly, recovery rate.

The initial series of three tagging experiments was performed in St. Mary's Bay in May and June 1974 (Table 1). Herring were captured in traps and bar seines. Three experiments were designed to test the effect of holding time on subsequent fish mortality by comparing returns from fish released on the day of capture with those held and released 1 or 2 d following capture. Five external tags were applied (carlin, disc and dangler, disc and disc, anchor, and dart) on the same day to compare suitability of tags.

In later years, experiments were conducted in other parts of Newfoundland to examine returns from applications of various external tags. In May 1978, herring were tagged in Trinity Bay from a bar seine, on the Southern Shore from a trap, and St. Paul's Inlet on the west coast from a trap to compare the effectiveness of employing anchor and streamer tags (Fig. 1). Variations on the comparison included two types of anchor tags (short anchor = 20 mm; normal anchor = 45 mm) and yellow and orange streamers. In six experiments, anchor and dart tags were applied following the preliminary results of the St. Mary's

TABLE 1. Summary of tagging experiments. Dates are day/month (day/month/year for anchor and dart comparisons).

Tag type	Area of release	Date of capture	Date of release	No. released	Total recovered
		1974 St. Mary's Bay comparisons			
Carlin	St. Mary's Bay	1/5; 14, 17/6	1/5; 14, 17/6	200	12
		8, 14/6	9, 15/6	150	7
		14/6	16/6	100	2
Disk and dangler	St. Mary's Bay	1/5; 14, 17/6	1/5; 14, 17/6	1113	72
		1/5; 8, 14/6	2/5; 9, 15/6	1000	42
		14/6	16/6	500	7
Disc and disc	St. Mary's Bay	14/6	14/6	300	18
		1/5; 8, 14/6	2/5; 9, 15/6	1000	59
		14/6	16/6	400	14
Anchor	St. Mary's Bay	1/5; 14, 17/6	1/5; 14, 17/6	450	34
		1/5; 8, 14/6	2/5; 9, 15/6	350	9
		14/6	16/6	200	4
Dart	St. Mary's Bay	1/5; 14, 17/6	1/5; 14, 17/6	900	36
		1/5; 8, 14/6	2/5; 9, 15/6	700	14
		14/6	16/6	400	7
		1978 Streamer and anchor comparisons			
Yellow normal anchor	Southern Shore	9, 10, 12/5	9, 10, 12/5	1000	124
	Trinity Bay	16, 17, 18/5	16, 17, 18/5	2500	28
	St. Paul's Inlet	29, 30/5	29, 30/5	3500	91
Orange short anchor	Southern Shore	9, 10, 12/5	9, 10, 12/5	500	43
	Trinity Bay	16, 17/5	16, 17/5	500	9
	St. Paul's Inlet	29, 30/5	29, 30/5	500	5
Yellow short anchor	Southern Shore	9, 10, 12/5	9, 10, 12/5	500	38
	Trinity Bay	16, 17/5	16, 17/5	500	12
	St. Paul's Inlet	29, 30/5	29, 30/5	500	6
Orange streamer	Southern Shore	9, 10, 12/5	9, 10, 12/5	400	48
	Trinity Bay	16, 17, 18/5	16, 17, 18/5	400	18
	St. Paul's Inlet	29, 30/5	29, 30/5	500	11
Yellow streamer	Southern Shore	9, 10, 12/5	9, 10, 12/5	500	53
	Trinity Bay	16, 17, 19/5	16, 17, 19/5	300	5
	St. Paul's Inlet	29, 30/5	29, 30/5	500	16
		Anchor and dart comparisons			
Anchor	Conception Bay	3/12/75	3/12/75	1350	42
		8/6/76	8/6/76	2000	56
	St. Mary's Bay	21, 23/4/76	21, 23/4/76	2325	313
	Southern Shore	27/5/76	27/5/76	600	11
		25, 26, 27/4/77	25, 26, 27/4/77	2000	190
	St. George's Bay	29/4/76	29/4/76	3400	128
Dart	Conception Bay	3/12/75	3/12/75	1000	64
		8/6/76	8/6/76	2000	6
	St. Mary's Bay	21, 23/4/76	21, 23/4/76	1500	250
	Southern Shore	27/5/76	27/5/76	300	9
		27/4/77	27/4/77	400	25
	St. George's Bay	29/4/76	29/4/76	3000	30

Bay studies. Herring were captrued and tagged in Conception Bay from purse seines in December 1975 and June 1976, in St. Mary's Bay from a purse seine in April 1976, on the Southern Shore from traps in May 1976 and April 1977, and in St. George's Bay from a purse seine in April 1976 (Table 1).

The results from tagging experiments of similar design (i.e. same tags applied in similar circumstances) were combined to facilitate analysis. Thus the results of the three experiments in St. Mary's Bay in 1974 were combined, the results of the three experiments examining streamer and two types of anchor tags were combined, and the results from the six experiments comparing dart and anchor tags were combined.

To minimize the initial influence of nonrandom distribution of marked fish in the population the number of recaptures was

TABLE 2. Summary of St. Mary's Bay tagging experiments conducted in 1974.

Tag type	Days held	All returns: No. recaptured	All returns: No. released	All returns: % recaptured	Returns adjusted for nonrandom distribution: No. recaptured	Returns adjusted for nonrandom distribution: No. released	Returns adjusted for nonrandom distribution: % recaptured
Carlin	0	12	200	6.0	12	200	6.0
	1	7	150	4.7	7	150	4.7
	2	2	100	2.0	2	100	2.0
Disc and dangler	0	72	1113	6.5	29	1070	2.7
	1	42	1000	4.2	29	987	2.9
	2	7	500	1.4	5	498	1.0
Disc and disc	0	34	600	5.7	16	582	2.8
	1	59	1000	5.9	6	947	0.6
	2	14	400	3.5	2	388	0.5
Anchor	0	34	450	7.6	20	436	4.6
	1	9	350	2.6	7	348	2.0
	2	4	200	2.0	3	199	1.5
Dart	0	36	900	4.0	25	889	2.8
	1	14	700	2.0	10	696	1.4
	2	7	400	1.8	6	399	1.5

adjusted by eliminating from the analysis tagged fish released and recaptured in the same year just prior to or during the fishery. This generally amounted to a 3-mo period. We assumed that these fish were not randomly mixed with untagged fish and their behaviour subsequent to release may have been quite different from untagged fish resulting in a bias in the number and pattern of recaptures following their release. Returns adjusted for effects of nonrandom distribution presented in Tables 2, 5, and 7 reflect this correction. The number of tagged fish at large in the population has been adjusted downwards by removing these biases.

To compare tag returns where experiments from different years and/or from different areas were combined (streamer–anchor comparison; anchor–dart comparison) we expressed recapture rates as the number of tags returned per thousand metric tons landed (Wheeler and Winters 1984) (i.e. we assumed catch to be proportional to fishing effort for all study areas). Recaptures per study area per year were combined, and then the annual catch for all study areas per year was employed to estimate returns per thousand metric tons landed. The final section in Tables 5 and 7 depict the results from performing this transformation on the return data.

To examine tagging rates for each of the different tag types, records of time taken to apply each tag were analyzed. For comparison, only those times for a two-man team were considered. For the 1974 St. Mary's Bay studies, results from one experiment were available. Data were available from the Trinity Bay and Southern Shore 1978 experiments utilizing anchor, short anchor, and streamer tags. Four tagging experiments in which anchor and dart tags were applied had sufficient information to estimate tagging rates.

Results

Initial Tagging Mortality

One purpose of the St. Mary's Bay experiments in 1974 was to determine the influence of the length of time herring were held in an enclosure on subsequent mortality of tagged fish. In three experiments, herring were tagged and subjected to the following treatments: (1) released on the same day of capture, (2) released on the first day after capture, and (3) released on the second day after capture. The percentage of recaptures from each day indicate that mortalities of tagging herring increased the longer fish were held before being released (Table 2). However, long-term recoveries of tagged herring beyond the initial year of release were not markedly lower than when tagged fish were released on the same day as capture (Table 3), i.e. as expected holding time results in significant short-term mortality only.

Winters (1977) demonstrated that the percent returns from tagged fish released 1 and 2 d after the date of capture, expressed as a ratio of the percent returns from fish tagged on the initial day of capture, were similar to the relative tagging survival estimates derived by a computational method described by Ricker (1958) and Dickie (1963). An initial check comparing the two methods indicated that ratios of percent returns gave reasonable estimates of relative survival estimates for comparing the effectiveness of prolonged captivity on the initial survival of tagged herring. In all comparisons with the external tags, except for the disc and dangler tag, holding fish beyond the day of capture dramatically decreased the chances for short-term survival of tagged herring (Table 4). Excluding the disc and dangler, herring held for 1 d had a median relative survival rate around 0.50, while those held for 2 d had a median value of approximately 0.33. In addition, those tag types such as carlin and disc and dangler that involved the greatest handling time (i.e. slowest application rate) tended to exhibit the greatest decrease in relative survival rates between the second and third day releases.

Sustained Tag Loss

Long-term tag loss (and/or tag-induced mortality) may be estimated from a comparison of mortality rates derived from catch curves based on annual tag recaptures and estimates of mortality during the same period from an independent source such as cohort analyses. For the St. Mary's Bay tagging

TABLE 3. Distribution of recaptures over time for five external tags applied in St. Mary's Bay in 1974.

Tag type	No. of days held	Year of recapture				
		0	1	2	3	Year unknown
Carlin	0	0	8	2	1	1
	1	0	6	1	0	0
	2	0	2	0	0	0
Disc and dangler	0	41	27	2	0	2
	1	15	21	4	1	1
	2	4	3	0	0	0
Disc and disc	0	28	4	0	0	2
	1	53	5	0	0	1
	2	12	2	0	0	0
Anchor	0	18	9	3	1	3
	1	2	4	2	1	0
	2	1	3	0	0	0
Dart	0	8	18	7	0	3
	1	4	4	4	1	1
	2	2	2	3	0	0
All types	0	95	66	14	2	11
	1	74	40	11	3	3
	2	19	12	3	0	0

TABLE 4. Relative survival rates for five external tag types comparing fish released on the same day of capture with those held and released after 1 and 2 d.

Tag type	Comparison	Ratio % recaptures adjusted for nonrandom distribution
Carlin	0 d vs 1 d	0.78
	0 d vs 2 d	0.33
Disc and dangler	0 d vs 1 d	1.07
	0 d vs 2 d	0.37
Disc and disc	0 d vs 1 d	0.21
	0 d vs 2 d	0.18
Anchor	0 d vs 1 d	0.43
	0 d vs 2 d	0.33
Dart	0 d vs 1 d	0.50
	0 d vs 2 d	0.54

experiments the lowest estimates of mortality were from the anchor and carlin returns (Fig. 2), whereas the highest mortalities were associated with the disc and disc and the disc and dangler tags. In all cases, however, mortality rates estimated from tag recapture data were substantially higher (Fig. 2; range: 1.04–1.97) than the comparable estimate ($Z = 0.40$) from cohort analysis (Moores et al. 1981). This implies a substantial long-term tag loss rate or tag-induced mortality for all tag types considered. Thus, even for the anchor tags, which yielded the lowest estimates of sustained tag loss, there would be less than 5% of the initial number effectively tagged (i.e. following the initial losses due to holding and handling) remaining 3 yr after released compared with an expected percentage of 30% based on equal mortalities for marked and unmarked fish in the population. From these data we conclude that none of the tag

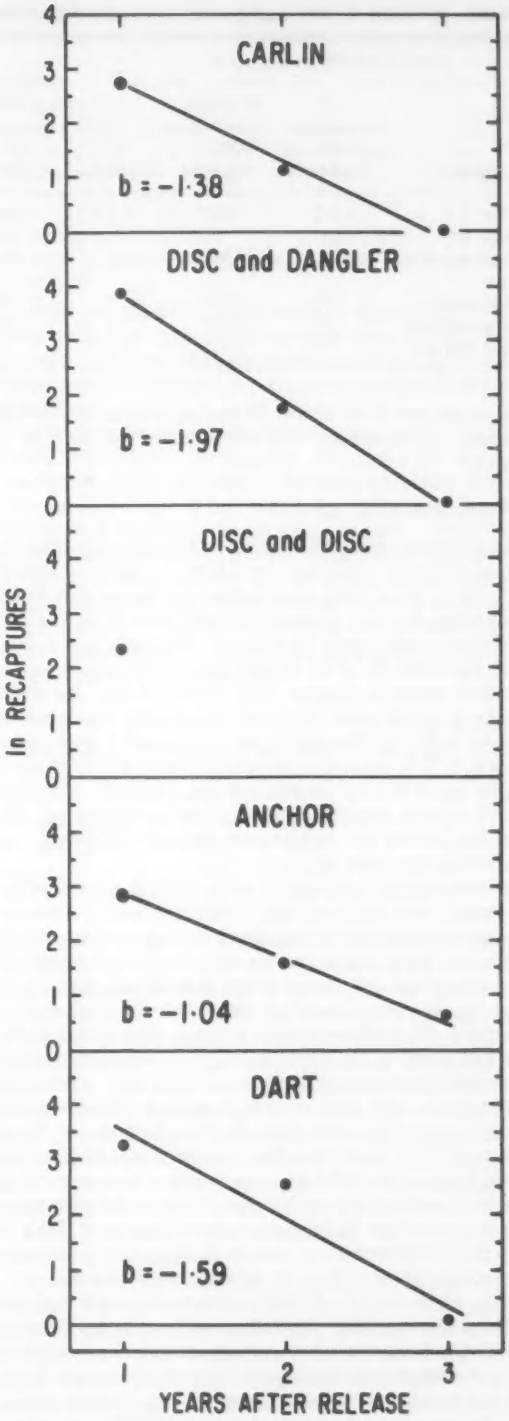

FIG. 2. Logarithm of annual recoveries of the five tags compared in the St. Mary's Bay experiments.

TABLE 5. Summary of three tagging experiments comparing anchor, short anchor, and streamer tags.

Tag type	All returns			Returns adjusted for nonrandom distribution			Returns standardized by catch		
	No. recaptured	No. released	% recaptured	No. recaptured	No. released	% recaptured	No. recaptured per 1000 Mt landed	No. recaptured per 1000 tags released	% recaptured
Anchor	243	7000	3.5	54	6811	0.8	15.3	14.3	1.4
Orange short anchor	57	1500	3.8	12	1455	0.8	3.3	7.0	0.7
Yellow short anchor	55	1500	3.7	8	1453	0.6	2.2	4.6	0.5
Orange Streamer	77	1300	5.9	18	1241	1.5	9.2	22.7	2.3
Yellow Streamer	74	1300	5.7	2	1228	0.2	0.8	1.7	0.2

types tested would be suitable for herring tagging experiments requiring tag recaptures over an extended period (3 yr or longer).

Selection of the Best Tag

Because prolonged holding times had a deleterious effect on herring, survival comparisons for selection of the most suitable tag in the St. Mary's Bay experiments were based upon results from herring that were released on the same day of capture. The highest return and hence the best tag type in this case was the carlin tag (Table 2). If we employ the ratio of returns method described earlier to compare effectiveness of tags, the results describe the relative survival of all four external tags compared with the carlin tag. The anchor tag had a relative survival rate that was 0.77 of the carlin, the dart tag was 0.47, the disc and dangler was 0.45, and the disc and disc was 0.47. Strictly on relative survival rates, the carlin tag remains the best tag, with the anchor second and the remaining three tags similar but less than 50% of the carlin tag.

In three tagging experiments where normal anchor, yellow and orange short anchors, and orange and yellow streamers were applied suggested that orange streamers were the best tag (Table 5). The normal anchor tag was second in recovery rate. Both the yellow and orange short anchors had very similar recovery rates, suggesting that colour made little difference in the returns. The yellow streamer was very poor with a percent recapture of only 0.2%. By examining the relative survival rates the magnitude of these differences is made clearer. The normal anchor had a 0.61 survival rate compared with the orange streamer, the orange and yellow short anchors were 0.30 and 0.22, respectively, and the yellow streamer was 0.09. Recovery rate data alone indicated that orange streamers were an excellent tag when compared with anchor tags. However, the prospects of long-term recoveries of streamers were not very good (Table 6). As with the attachment tags used in St. Mary's Bay, streamers were not recovered beyond the first year after release.

In the six experiments where only dart and anchor tags were applied anchor tags were more effective than dart tags (Table 7). Anchor tags had a return rate of 3.5% compared with 2.9% for dart tags when returns were adjusted for nonrandom distribution and standardization by catch (Table 7). The relative survival rate of dart tags was 0.83 of anchor tags. In these comparisons where only two tags were applied and in large number, the small but higher recovery rate of anchor to dart tags was similar to results estimated from the St. Mary's Bay experiments. The long-term recoveries of both tags of up to 3 yr after the release

TABLE 6. Distribution of recaptures over time for normal anchor, short anchor, and streamer tags.

Tag type	No. days held	Year of recapture 0	1	2
Normal anchor	0	207	29	7
Orange short anchor	0	49	7	1
Yellow short anchor	0	50	6	0
Orange streamer	0	73	4	0
Yellow streamer	0	72	2	0

year substantiated the results of the St. Mary's Bay studies (Table 8).

A significant factor to consider in mark–recapture programs is the ease of application of various tag types. For studies on migration and stock delineation it is often a good strategy to tag as many fish as possible. To examine this factor, we chose to use the rate of tagging as an indicator of how easily a specific tag may be applied. Presumably the higher the tagging rate the easier a tag can be applied. In all comparisons, the times refer to one two-man tagging team. The highest rates of application were achieved with the normal anchor and short anchor tags (Table 9); carlin and the disc and disc were the slowest to apply. Streamers fared slightly higher than the disc and dangler tags in this respect. Generally, anchor and dart tags were applied significantly faster than all other external tag types described in this report.

Discussion

Based upon the criteria reported thus far, we conclude that anchor and dart tags are the most suitable tags to use in herring tagging experiments. However, other considerations may be used by the researcher to make a final selection.

Referring to the four criteria we posed at the beginning of the study, our analysis has indicated that all external tags considered herein meet the requirements to be recognizable to commercial fishermen and fish plant workers. Two tags, the disc and disc (Table 2) and streamer (Table 6), may be disregarded for any studies that require returns several years

TABLE 7. Summary of six tagging experiments comparing anchor and dart tags.

Tag type	All returns: No. recaptured	All returns: No. released	All returns: % recaptured	Returns adjusted for nonrandom distribution: No. recaptured	Returns adjusted for nonrandom distribution: No. released	Returns adjusted for nonrandom distribution: % recaptured	Returns standardized by catch: No. recaptured per 1000 Mt landed	Returns standardized by catch: No. recaptured per 1000 tags released	Returns standardized by catch: % recaptured
Anchor	740	11 675	6.3	313	11 248	2.8	62.5	34.7	3.5
Dart	384	8 200	4.7	150	7966	1.9	21.7	28.7	2.9

TABLE 8. Distribution of recaptures over time for anchor and dart tags.

Tag type	No. of days held	Year of recapture: 0	1	2	3
Anchor	0	593	131	15	1
Dart	0	281	77	24	2

TABLE 9. Rate of application of the seven tag types considered in the optimum tag selection comparisons.

Tag type	No. tags applied	Elapsed time (h)	Tagging rate (tags/h)
Carlin	100	4.17	24
Disc and disc	300	3.25	92
Disc and dangler	500	4.25	118
Streamer	1 600	9.58	167
Dart	5 625	23.75	237
Short anchor	2 000	7.25	273
Anchor	10 911	37.33	292

after release, since no recoveries were made after the first year of release. Indirectly, using return rates and duration of tagged fish remaining in the population one may be able to reject some tags due to either a significant mortality of tagged fish or a high shedding rate. Carlin tags had the highest survival rate, with the anchor tag slightly lower. The disc and dangler, disc and disc, and dart tags had survival rates half that of the carlin, and may be eliminated on this basis. The use of relative survival rates in this manner demonstrated that of all the tags considered the carlin tag had the least harmful influence on herring. From comparison between the anchor and streamer tags (Table 7), anchor tags had a relative survival rate of 0.61 of that of orange streamers. However, the results of this comparison require further study, since there is a large unexplainable discrepancy between orange and yellow streamers. Further, the lack of any returns from the first year of release (Table 6) for either streamer type would suggest that tagging mortalities, excessive predation on streamer-tagged fish, or shedding of streamer tags may have occurred compared with fish tagged with anchor tags. The fourth consideration was that tags be easy to apply. Owing to the high handling mortalities that occurred in our St. Mary's Bay experiments and also described previously by Winters (1977) and Jensen (1955), it is imperative that tags be applied quickly on the day of capture. Tags that can be applied rapidly also result in a large proportion of the population being tagged, which should result in a higher number of tags being returned. This is especially important when the information from the pattern of returns is used for making inferences about migrations and stock delineation.

To illustrate the importance of the various criteria in an actual tagging experiment, we consider the choice of tag for a hypothetical mark–recapture experiment involving herring. The experimenter would like to determine the migration pattern and degree of homing to the spawning area over several years. The opportunity to tag the fish is limited to 5 d, with approximately 5 h available per day for the actual tagging operation once the herring are sighted and captured. One two-man tagging team is available. Using the return rates in Table 2, the number of years for recapture in Table 3, and the tagging rates in Table 9, we obtain the following results:

Tag type	No. marked	No. returned	No. years over which recaptures occur
Carlin	600	36	3
Disc and dangler	2950	80	2
Disc and disc	2300	64	1
Anchor	7300	336	3
Dart	5925	166	3

Thus, based upon our estimates of the relevant tagging parameters one would utilize the anchor tag for this study. Clearly the higher survival rate of carlin tags over the anchor tag is compensated by the anchor tag having a very much higher application rate. Because the researcher is concerned with the number of returns, a tag that can produce a high number of returns will give more information on migration and stock patterns even though the tags may cause higher mortality of tagged fish. With regard to the choice between anchor and dart tags, in addition to its higher application rate there is no setting up time involved with anchor tags whereas dart tags must be loaded into individual needles prior to the actual tagging operation. This time cost has not been included in our estimates of dart tag application rates.

Thus we conclude that among the seven types of external tags compared, the most suitable is the anchor tag. Returns are made over several years and its very high application rate insures a large number of returns despite a lower initial survival rate of tagged fish compared with those with carlin tags. From the results of the comparison between the anchor and dart tag experiments and the small amount of time required to set up for tagging with anchors compared with the dart tag, we feel that the anchor tag remains the most effective external tag to use to address problems in migration and stock discrimination in herring.

This report has also provided further evidence in support of Winters (1977) and Jensen (1955) who have shown that mortalities of tagged herring can be greatly reduced by tagging fish on the same day of capture. In addition the much higher sustained mortality rates (Fig. 2) associated with tagged herring that had survived the initial holding mortalities indicate that the tag itself has the apparent effect of reducing the survival rate of tagged herring compared with the untagged herring. In our studies, tag loss may have resulted from tag-induced mortality and/or shedding of tags. From holding anchor tagged herring in captivity for 6–18 mo, Hay (1981) concluded that tag shedding increased with time. If this were true in our study then our tag-induced mortalities may be overestimated. Although we can not differentiate the causal mechanism, the overall effect is higher loss of tagged fish than expected from mortality estimates derived from cohort analysis.

Thus none of the seven external tags are appropriate for studies requiring long-term tag returns for more than 2 yr.

Finally, there are differences in the return rates as a result of changes due to adjustments for nonrandom distribution (Tables 2, 5, and 7) and standardization according to catch (Tables 5 and 7). Different conclusions would have been drawn had the original return data been used without consideration of these biases. For example, in Table 2 there would have been a significant difference in the order of how effective the tags were based upon return rates. Similarly, in Table 5 the differences between streamer and anchor tags would not have been so pronounced as shown by the standardized data. These results suggest that studies that examine return rates over different years and that combine results from different areas must consider some method to minimize potential biases in the raw data before proceeding with the analysis.

In summary, for any mark–recapture program, the researcher must consider what factors are involved in choosing the appropriate tag to apply. For herring studies, the tagging rate, the number of years in which recaptures will be made following release, and the recovery rate are all important criteria to optimize before proceeding with the experiment.

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Demographic Consequences of Precocious Maturation of Atlantic Salmon (*Salmo salar*)

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Myers, R. A. 1984. Demographic consequences of precocious maturation of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 41: 1349–1353.

Detailed records of outgoing smolts of Atlantic salmon (*Salmo salar*) and estimates of the parr population that produced them were used to study the demographic conequences of male precocious sexual maturation in southwest Newfoundland. Approximately 80% of males matured precociously in the stream. Annual survival probability of a male that reproduces precociously is reduced by a median 44%. Precocious maturation delays smolting; the probability of smolting in the second year for a male precocious parr is approximately 13% of that of a female. The economic loss due to precocious maturation is large; 60% of the adult male salmon production is lost due to precocious maturation.

Des données détaillées sur des saumoneaux de saumon de l'Atlantique (*Salmo salar*) descendant vers la mer et des estimations des populations originales de tacons ont été utilisées pour étudier les conséquences démographiques de la maturation sexuelle précoce des mâles au sud-ouest du Groenland. Environ 80 % des mâles sont arrivés précocément à maturité dans le milieu limnicole. La probabilité annuelle de survie d'un mâle dont la reproduction est précoce est réduite de 44 %, chiffre médian. Une maturation précoce retarde la smoltification ; ainsi, la probabilité qu'un tacon mâle précoce devienne un saumoneau au cours de la deuxième année est d'environ 13 % de celle de la femelle. La perte économique due à la maturation précoce est élevée : 60 % de la production de saumons mâles adultes est ainsi perdue.

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In many anadromous salmonids, males may become sexually mature before migrating to the sea (*Salmo salar*, Jones 1959; *S. gairdneri*, Schmidt and House 1979; *Oncorhynchus tshawytscha*, Robertson 1957; *O. masou*, Utoh 1976; *O. nerka*, Hanson and Smith 1967; *Salvelinus fontinalis*, Power 1980). These precocious parr dart in close to spawning adult pairs and shed sperm in competition with the adult males (Jones 1959).

There is clear evidence that some of the precocious parr migrate to the sea as smolts (Leyzerovich and Mel'nikova 1979; Dalley et al. 1983) and some remain in the river for at least 1 yr after maturing (Mitans 1973; Gibson 1983). Furthermore, there is evidence that male parr that mature precociously incur a higher mortality than those that do not (Dalley et al. 1983). There are no quantitative estimates for the proportion of precocious parr that do not smoltify or the reproduction–mortality associated with precocious maturation. The purpose of this paper is to provide such quantitative estimates and to investigate the demography of the Atlantic salmon parr.

Methods

The approach taken in this paper is to compare the numbers of female, immature male, and precocious male parr in an age-class with the number of female, immature male, and precocious male parr and smolt the following year to determine the patterns of maturation, mortality due to maturation, and smolting that are consistent with the observed numbers at age in the 2 yr.

The Data

Murray (1968a, 1968b, 1968c, 1968d) described the Little Codroy River salmon demographic study in detail. The data discussed in this paper are drawn from records of parr and smolt sampled during this project, which was under the direction of A. R. Murray.

My analysis combines data from two separate phases of the study: sampling to estimate parr population densities in the river and sampling of the smolt run at the river outlet. Parr were collected from 38 sampling stations in the Little Codroy River drainage basin in August and September of each year using an electrofishing apparatus (Murray 1958). Each station was fenced with a net before electrofishing began. Multiple sweeps were made with the electrofisher until no more fish were caught. The estuary and deep pools (1 m depth) were sampled using a modified 10-m trawl, but very few parr were found in these areas. Over 500 parr were sampled each year except for 1959 when 300 parr were sampled. Although the sampling was begun in 1956, sufficient stations were not established to provide representative estimates of the parr population until 1959 when approximately 1.7% of the stream bed was sampled (Murray 1961).

Data from 1959 to 1963 appear to be quantitatively reliable

TABLE 1. Abundance data used for the analysis.

Parr									
Year	m_1^i	m_1^p	m_2^i	m_2^p	m_3^i	m_3^p	f_1	f_2	f_3
1959	8307	21 770	4010	14 323	286	3724	19 192	16 041	1145
1960	5799	20 085	919	3 819	0	778	23 904	4 526	424
1961	6826	11 377	1327	6 637	95	85	14 691	6 542	0
1962	4349	14 827	845	7 595	121	482	18 687	7 716	1206
1963	4124	8 248	546	3 760	0	910	12 069	3 457	121

Smolt									
Year	M_2^i	M_2^p	M_3^i	M_3^p	M_4^i	M_4^p	F_2	F_3	F_4
1960	304	76	895	934	19	29	1505	4039	48
1961	716	179	447	652	13	153	3556	2162	179
1962	926	357	489	688	13	26	3308	2368	13
1963	600	320	200	660	0	60	3303	2142	40

and were the only data used here. The length, weight, and stage of sexual maturation were recorded for each individual parr, and scales were sampled and analyzed to estimate age and to back-calculate growth rates. Maturation in male parr was assayed by examining the gonads of the parr and categorizing them as in Jones and Orton (1940). Basically, a parr could be identified as immature (Jones' stage I), maturing (Jones' stages II–IV), mature (Jones' stages V–VI), or spent (Jones' stage VII). No spent male parr were recorded; samples were not taken after October, the time of spawning. No sexually mature female parr were captured during the study.

Smolts were collected and counted at a smolt fence placed at the mouth of the Little Codroy River. The counts for the total number of smolts were very accurate. The smolt run lasted approximately 2 mo; 50% of the smolts were counted by approximately June 20 of each year (Murray 1968c). The efficiency of the smolt fence was substantiated by a mark–recapture experiment (Murray 1968c). A sample of the smolt run was taken throughout the run and analyzed in the same manner as described for the parr (this sample was over 500 fish for each year used).

Total numbers of parr and smolts of each age- and sex-class were estimated by multiplying the total estimated number of parr and smolts by the proportion of each sex–age-class in the subsample (Table 1).

Growth during the first year was inferred from scale annuli determined by standard criteria (Berg and Grimaldi 1967). Annulus radii were measured from the focus along the longest oral radii (Tesch 1968).

Demographic Model

I will estimate the survival and the proportion of parr that mature or become smolt at each age from data on abundances of parr and smolts. The notation for the abundances and the parameters are described in Table 2.

If all transitions from parr to smolt and from immature to mature parr could be identified, standard procedures could be used to estimate the transition probabilities between states (Billingsley 1961). Unfortunately, this is not the case. It was not possible by visual examination of the gonads to distinguish male parr that mature for the first time from those that matured previously. It is possible to estimate the proportion of mature parr that remain in the river by assuming that the survival of immature male parr is the same as the survival of female parr. If this assumption is not made, there is not sufficient information to distinguish between greater mortality due to precocious maturation and the proportion of precociously mature parr that remain in the river an extra year.

There is evidence that the assumption of equal survival for immature males and females is reasonable: (i) populations with very few precociously mature males have a 1:1 sex ratio (Schiefer 1971) and (ii) hatchery and wild populations maintain a 1:1 sex ratio until the age that precociously mature males appear (Vladimirskaya 1958; Mitans 1973; Dalley 1978). The sensitivity of my conclusions to this assumption was checked.

The mortality rate of precocious parr is different from that of other parr (Myers 1983). Since the precocious parr were censused less than 2 mo before mating occurred, it is assumed that the mortality due to precocious maturation took place during spawning or between spawning and the next census.

Because the smolt census is 2 mo earlier than the parr census, I initially assume that the survival of parr from the census in August–September to the smolt census the following June is 1.2 times the survival of parr from one year to the next. No parr were

TABLE 2. Basic parameters and variables in the model.

δ_j	Survivorship during 1 yr for immature male or female parr of age j
$\delta_{j,p}$	Survivorship during 1 yr for precociously mature male parr of age j
f_j	Number of female parr of age j
F_j	Number of female smolts of age j
m_j^i	Number of immature male parr of age j
m_j^p	Number of precociously mature male parr of age j
M_j^i	Number of immature male smolts of age j
M_j^p	Number of precociously mature male smolts of age j
p_i	Proportion of immature male parr of age i that would become precociously mature at age $i + 1$ if there was no natural mortality
q_i	Proportion of immature male parr of age i that would become smolt at age $i + 1$ if there was no mortality
r_i	Proportion of precocious male parr of age i that would become smolt at age $i + 1$ if there was no mortality
s_i	Proportion of female parr of age i that would become smolt at age $i + 1$ if there was no mortality

found in the river that had regressed from precocious to nonreproductive (such males would have been detected by the examination of their gonads in the same manner that spent smolts were detected). Thus, I assume that all precocious male parr that do not smoltify the following year remain precociously mature the following year. That is, precocious male parr either remain in the river as precocious parr, die, or become smolts.

The following model was thus posed for the change in abundance of parr:

$$
\begin{aligned}
m^i_{j+1} &= [1 - (p_j + q_j)]\delta_j m_j^i \\
m^p_{j+1} &= p_j\delta_j m_j^i + (1 - r_j)\delta_{jp} m_j^p \\
M^i_{j+1} &= 1.2q_j\delta_j m_j^i \\
M^p_{j+1} &= 1.2r_j\delta_{j,p} m_j^p \\
f_{j+1} &= (1 - s_j)\delta_j f_j \\
F_{j+1} &= 1.2s_j\delta_j f_j
\end{aligned} \qquad (1)
$$

for $j = 1, 2$ (see Table 2 for an explanation of the notation). The following constraints were assumed:

$$
\begin{aligned}
&0 \leq p_j \leq 1 \text{ for } j = 1, 2 \\
&0 \leq q_j \leq 1 \text{ for } j = 1, 2 \\
&0 \leq r_j \leq 1 \text{ for } j = 1, 2 \\
&0 \leq s_j \leq 1 \text{ for } j = 1, 2 \\
&p_j + q_j \leq 1 \text{ for } j = 1, 2 \\
&0 \leq \delta_j \leq 1 \text{ for } j = 1, 2 \\
&0 \leq \delta_{j,p} \leq 1 \text{ for } j = 1, 2.
\end{aligned} \qquad (2)
$$

The rarity of age 4 and older parr and smolts precluded reliable parameter estimates after age 3.

Note the following about the model: (i) Immature male parr of ages 1 or 2 may remain as immature parr, may become precociously mature parr, or may become smolt the next year; (ii) precociously mature parr of ages 1 or 2 may remain in the river as precocious parr or may become smolts the next year; (iii) precocious male parr remain precocious for a second year if they remain in the river; (iv) mortality rates are the same for immature males and female parr.

Results

Fitting Models to the Data

The model was fitted to the data for each year separately because there is evidence that the age at smoltification is not constant (Chadwick 1981). For any year the 12 equations in (1) contain 12 unknown parameters that are to be estimated. Thus, there is exactly enough information to uniquely specify each of the parameters. This is done by a simple algebraic rearrangement of the equations in (1) to sequentially estimate δ_j, s_j, q_j, p_j, $\delta_{j,p}$, and r_j for each age.

Initially, the equations in (1) were fitted to the data for the 3 yr without imposing inequality constraints (Eq. 2). The 12 parameters in the model were estimated separately for each of the four sets of data. The only inequality constraint that was violated in any of these four fittings was the parameter p_2 (the proportion of immature 2-yr-old parr that become precocious at age 3), which was less than zero (-0.28) for the 1960–61 transition. That only one of the inequality constraints was violated in the four data sets is evidence that the biological assumptions of the model are reasonable, and that the data are sufficiently accurate to yield meaningful rate parameters. The most reasonable biological interpretation of the inequality constraint being violated is that very few or no parr mature for the first time at age 3, and sampling errors resulted in the unconstrained estimates being less than zero. Next, the model was fitted to the 1960–61 data with the inequality constraint $p_2 \geq 0$ imposed (Table 3). The parameter q_2 was then over identified, i.e. there are two possible parameter estimates for it. The mean value of the two possible estimates was chosen as the appropriate estimate of q_2 in the 1960–61 transition.

TABLE 3. Rate parameters estimated in the model. The asterisk indicates that an inequality constraint, $p_2 \geq 0$, was imposed.

Year	Age (j)	p_j	q_j	r_j	s_j	δ_j	$\delta_{j,p}$
1959–60	1	0.53	0.1	0.025	0.22	0.3	0.12
	2	0.21	0.79	0.57	0.89	0.24	0.095
1960–61	1	0.17	0.26	0.023	0.31	0.4	0.32
	2	0*	0.88	0.85	1	0.4	0.17
1961–62	1	0.67	0.16	0.065	0.26	0.72	0.41
	2	0.18	0.63	0.61	0.62	0.49	0.14
1962–63	1	0.27	0.35	0.074	0.44	0.33	0.24
	2	0.2	0.8	0.39	0.94	0.25	0.19

A probable reason for the high survivorship estimates for the 1961–62 parameters is that the parr population is underestimated in 1961. In 1961, the water levels were unusually low (Murray 1968d) and parr probably migrated into deeper pools that were not consistently sampled because few parr were found there previously (R. F. Burfitt, Department of Fisheries and Oceans, P.O. Box 5667, St. John's, Nfld. A1C 5X1, pers. comm.).

Standard errors are easily calculated for the results if it is assumed that all samples were strictly random, there is no model error (e.g. immature male mortality is exactly the same as female mortality in all years), and there are no errors in the estimation of the total parr population size. The resulting standard errors are quite small but are misleading and will not be reported here. However, it is shown in the next section that the parameter estimates are robust to the realistic violations of the above assumptions.

Although it is not possible to make quantitatively reliable inferences for the parr that remain in the river after age 2, qualitative information can be inferred. In particular, the rarity of parr older than age 3 (4 of the 6000 individuals sampled) implies that nearly all the parr become smolts, or die by age 4. This implies that few or no precocious parr remain permanently in the river as occurs in the Black Sea (Barach 1962) and the Matamec River in Quebec (Gibson 1983).

Sensitivity Analysis

An error in estimating the proportion of each age- and sex-class was more likely for the parr than for the smolts because of the difficulty in obtaining random samples from a stream. The accuracy of the estimates of the proportions of the sex- and age-classes can be checked by comparing the number of males and females of age 1. Since there is a 1:1 ratio at birth (Dalley et al. 1983), this ratio should also be 1:1. There are more male than female 1-yr-old parr in the samples (Table 1). The sensitivity of the estimates was checked under two hypotheses: (i) precocious male parr were systematically overestimated and (ii) immature male parr were systematically overestimated. The

analysis was repeated using the first hypothesis by modifying the parr abundances such that for a given year:

$$(3)\quad m_j^{\,p} = m_j^{\,p} \times \frac{f_1 - m_1{}^i}{m_1{}^p}$$

for $j = 1, 2$. The abundances were modified to check the second hypothesis such that

$$(4)\quad m_j^{\,i} = m_j^{\,i} \times \frac{f_1 - m_1{}^p}{m_1{}^i}$$

for $j = 1, 2$. These transformations make the sex ratio 1:1 for 1-yr-old parr. The modified parameter estimates are not greatly affected (Table 4) by either change.

If parr abundance is uniformly under- or over-estimated, then the resulting parameter estimates are changed (Table 4). Note that the estimate of the mortality due to precocious maturation ($\delta_j - \delta_{j,p}$) is relatively robust to such errors.

The model's sensitivity to the assumption that immature male and female mortality are equal was investigated. Immature male mortality was assumed to be 20% higher than female mortality. No parameter estimate in Table 3 was greatly changed. Similar results were found for the case of higher male mortality.

If mortality during the smolt run is different from the mortality for parr that remain in the stream, then the assumption that survival from parr census to smolt census may be different from 1.2 times the survival from parr census to parr census. Reasonable changes in this factor of 1.2 have only a minor effect on the rate parameter estimates (Table 4).

Analytical Results

The following patterns emerge:

(i) Approximately 80% of male parr were observed to mature precociously.

(ii) The proportion of 1-yr-old precocious parr that smoltify the following year is small, around 5%. This proportion increases to close to 50% for 2-yr-old mature parr and almost 100% for 3-yr-old mature parr.

(iii) Annual survival for male parr that mature precociously is significantly reduced (one-sided sign test, $p < 0.05$). The median mortality due to precocious maturation per year ($\delta_j - \delta_{j,p}$) was 0.18 (95% confidence limit 0.08–0.27), which resulted in a median 44% reduction of annual survival. Since most precocious parr spend an extra year (or more) in the river, their total survival as parr is further reduced. This calculation of the reduction in survival probably underestimates the total mortality due to precocious maturation because precociously mature males may have a lower survival once they enter the sea. Although there is no direct evidence for this, Dalley et al. (1983) found that the condition factor of spent precocious smolts was significantly reduced.

(iv) The parameter that varies the most between year-classes is p_1, the proportion of 1-yr-old immature male parr that mature as 2-yr-olds. Note that p_1 was high when smoltification success was low (i.e. q_1 and s_1 were low, Table 3). Because both precocious maturation and smoltification have been related to growth rate (Alm 1959; Bailey et al. 1980), the year-to-year variation in growth rate was investigated. Growth was slower in 1960 and 1961 than in 1962 and 1963. The mean first-year growth rate as measured by the radius of the first scale annulus was 8.75 mm in 1958, 9.95 mm in 1959, 9.027 mm in 1960, 9.131 mm in 1961, 10.217 mm in 1962, and 11.01 mm in 1963. The between-year growth differences are significantly different (ANOVA; $p < 0.0001$). The relatively slow growth in 1958 and 1960 may have been responsible for the relatively small percentage of 1-yr-old precocious males in 1959 and 1961 (Table 1) and the high percentage of males from these cohorts that matured for the first time as 2-yr-olds. Although the growth difference between years was highly significant the relationship between growth and the age that a parr is precocious cannot be verified with the data at hand. However, the observed variation in growth rate is evidence that the between-year variation in the parameters in Table 3 is real and not a sampling artifact.

(v) Precocious maturation is usually expressed by age 2, i.e. $p_2 \approx 0$ (Table 3).

(vi) Mortality is apparently higher for older ages for both precociously mature and immature individuals (Table 3; one-sided sign test, $p < 0.05$). This is possibly an artifact due to the inadequate sampling of older parr. If this difference in mortality is real it would explain the fact that all precocious parr that survive eventually become anadromous. That is, mortality may increase with size (possibly due to size selective predation) such that it is not advantageous for precocious parr to remain in the river after age 3.

TABLE 4. Model sensitivity analysis using data for 1962–63. The resulting rate parameters are changed as indicated under the following assumptions: (1) mature male parr abundances modified by Eq. 3, (2) immature male parr abundances modified by Eq. 4, (3) parr abundance assumed to be 20% underestimated, (4) immature male survival per year assumed to be 90% of female survival, (5) survival from parr to smolt assumed to be the same as survival from parr to parr, and (6) survival from parr to smolt assumed to be 1.4 times survival from parr to parr.

Assumption	Age (j)	p_j	q_j	r_j	s_j	δ_j	$\delta_{j,p}$
1	1	0.27	0.35	0.076	0.44	0.33	0.24
	2	0.2	0.8	0.4	0.94	0.25	0.19
2	1	0.22	0.39	0.071	0.44	0.33	0.25
	2	0.1	0.9	0.38	0.94	0.25	0.19
3	1	0.28	0.31	0.062	0.4	0.31	0.24
	2	0.21	0.79	0.34	0.92	0.21	0.18
4	1	0.19	0.39	0.071	0.44	0.33	0.25
	2	0.11	0.89	0.38	0.94	0.25	0.19
5	1	0.27	0.38	0.088	0.49	0.36	0.25
	2	0.19	0.81	0.43	0.95	0.29	0.2
6	1	0.28	0.32	0.063	0.41	0.31	0.24
	2	0.21	0.79	0.35	0.93	0.21	0.18

Discussion

The role of precocious maturation has been virtually ignored in demographic models of Atlantic salmon (but see Power 1969) even though precocious maturation results in the loss of approximately 60% of the male salmon production in Newfoundland. At least in the Little Codroy River this behaviour should not be considered abberrant, since 80% of the males are "precociously" mature as parr. The principal goal of this paper was to disentangle the mortality due to precocious maturation from the proportion of mature males that remain in the stream for an extra year. The methods used here allow these two parameters to be estimated for the first time.

These two parameters are very important for understanding the evolution of precocious maturation because they allow the

cost (maturity) and the benefits (number of eggs fertilized) of precocious maturation to be assessed. Precociously mature male parr are almost one half as likely to survive 1 yr in the stream as immature males or females. Furthermore, the energy used for precocious maturation greatly delays the age at which precocious male parr become smolts. It is estimated that from 2 to 7% of 1-yr-old precocious male parr become smolts in their second year. The comparable figures are 10–35% for immature males and 22–44% for females. These conclusions hold if the model assumptions are modified within a reasonable range; e.g. the results hold if immature male and female mortality are not equal. Thus, the increased mortality rate due to precocious maturation is high. Precocious maturation is only evolutionarily stable (Maynard Smith 1974) under such high mortality if the adult mortality is also great (Myers 1983; see Myers and Doyle (1983) for evolutionary methods of estimating natural mortality). Myers (1983) showed that changes in the proportion of precociously maturing parr can have a profound effect on the yield of a fishery due to the high mortality associated with precocious maturation and that man-induced changes in the proportion of precociously maturing parr can result from an evolutionary response to fishing pressure.

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Extracellular Protease, Extracellular Haemolysin, and Virulence in *Aeromonas salmonicida*

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Hackett, J. L., W. H. Lynch, W. D. Paterson, and D. H. Coombs. 1984. Extracellular protease, extracellular haemolysin, and virulence in *Aeromonas salmonicida*. Can. J. Fish. Aquat. Sci. 41: 1354–1360.

We have examined the possibile relationships between extracellular protease and haemolysin and virulence in *Aeromonas salmonicida*, and the possible plasmid-encoded origin of these extracellular enzyme activities. Variants, isolated from different strains, showed simultaneous loss of both protease and haemolysin. The frequency of spontaneous loss from one strain (0.1–0.4%) as well as the frequency of induced loss from four strains (0.3–18%) treated with ethidium bromide suggested a plasmid-encoded origin for these enzymes. However, plasmid analyses showed no detectable loss of plasmids correlated with the loss of the extracellular activities. There was no change in the LD_{100} when fish were exposed to one virulent strain or its protease, haemolysin-negative variants indicating that these extracellular activities are not necessary for virulence or pathogenicity in the acute form of furunculosis. Furthermore, both protease, haemolysin-negative and protease, haemolysin-positive clones isolated from a second virulent strain treated with ethidium bromide were avirulent (LD_{50} increased >4 logs). Thus, attenuation of *A. salmonicida* can occur without detected loss of the extracellular enzymes, plasmids, or the A-layer.

Les auteurs examinent les relations possibles entre la protéase et l'hémolysine extracellulaires et la virulence de *Aeromonas salmonicida* et étudient la possibilité d'un codage plasmidique responsable de ces activités enzymatiques extracellulaires. Des variants, isolés de souches différentes, ont présenté une perte simultanée de protéase et d'hémolysine. La fréquence de la perte spontanée chez une souche (de 0,1 à 0,4 %) ainsi que la fréquence de la perte induite chez quatre souches (de 0,3 à 18 %) traitées avec du bromure d'éthidium portent à croire à une origine codée par les plasmides. Toutefois, des analyses n'ont révélé aucune perte décelable de plasmides reliée à la perte des activités extracellulaires. Aucune variation de la DL_{100} n'a été notée chez des poissons exposés à une souche virulente ou à ses variants protéase et hémolysine négatifs, ce qui porte à croire que ces activités extracellulaires ne sont pas essentielles à la virulence ou à la pathogénécité de la forme aiguë de la furonculose. De plus, des clones protéase et hémolysine négatifs et des clones protéase et hémolysine positifs isolés d'une seconde souche virulente traitée avec du bromure d'éthidium étaient non virulents (DL_{50} augmentée de plus de 4 logarithmes). Donc, l'atténuation de *A. Salmonicida* peut avoir lieu sans perte décelable d'enzymes extracellulaires, de plasmides ou de couche-A.

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Aeromonas salmonicida is the etiological agent of furunculosis, currently one of the most important diseases affecting salmonids in both wild and cultured populations. The virulence mechanism(s) of *A. salmonicida* is not completely understood (Paterson 1981). Udey (1978) and Ishiguro et al. (1981) found that a major requisite for virulence of the bacterium is the A-layer, surrounding the outer membrane. Loss of the A-layer under certain cultural conditions results in attentuation of the bacterium.

This microorganism produces several extracellular products, which possess haemolytic, leukocytolytic, and proteolytic activities (Ellis et al. 1981), and which may also be involved in virulence and/or pathogenicity. The injection of cell-free preparations containing these extracellular products into fish resulted in pathology similar to furunculosis infections (Klontz et al. 1966; Fuller et al. 1977; Sakai 1977; McCarthy and Roberts 1980; Munro et al. 1980; Cipriano 1980; Ellis et al. 1981). Based on findings using crude extracellular growth products, Sakai (1977) indicated that production of an extracellular protease was required for furunculosis, and that it was the major pathogenic element produced by *A. salmonicida*, in agreement with the results of Cipriano et al. (1981). Cipriano et

al. (1981) reported that the pathogenicity of the organism was not associated with leukocytoxicity, as the leukocytolytic activity from different isolates varied and was unrelated to virulence. Little is known about the role of haemolysin, although two haemolytic activities have been identified (Titball and Munn 1981).

In several bacterial pathogens, properties associated with virulence and/or pathogenicity are plasmid-encoded (Crosa et al. 1977; Lopez-Alvarez and Giles 1980; Ben-Gurion and Shafferman 1981; Sansonetti et al. 1982), including some extracellular haemolysin (Waalwijk et al. 1982) and protease (Blascher and Solberg 1981) activities. Although *A. salmonicida* strains carry several plasmids (Toranzo et al. 1983), and while conjugation studies have confirmed the presence of R factors (Aoki et al. 1971), the functions of other plasmids are unknown (Ishiguro et al. 1981; Toranzo et al. 1983).

The purpose of the present study was to examine the possibility that extracellular protease and haemolysin produced by *A. salmonicida* strains are plasmid-encoded and to determine whether these extracelluar enzymes are necessary for virulence in this bacterium.

Materials and Methods

Bacteria, Growth Conditions, and Isolation of Bacterial Variants Lacking Extracellular Protease (prt^-) and Haemolysin ($haem^-$) Activities

Wild-type *A. salmonicida* strains were obtained from various sources (Table 1). Cultures were maintained and protease activity was detected on skim milk agar (10% w/v) overlay plates containing brain heart infusion (BHI) agar (Difco Laboratories, Detroit, MI) as a basal medium. Haemolysin activity was detected on blood agar (10% v/v) overlay plates (BHI) using human or fresh sheep's blood.

Lyophilized cultures were suspended in BHI broth (10 mL) and incubated at 22°C at 150 rpm on a psychrotherm controlled environmental shaker (model G-26, New Brunswick Scientific, New Brunswick, NJ) for 48–96 h. Serial 10-fold dilutions in saline (0.85% w/v) were spread on skim milk and blood agar overlay plates. After 72 h of incubation at 22°C, plates were examined for the presence of prt^- and $haem^-$ colonies. Selected prt^+, $haem^+$ colonies were inoculated into BHI broth, incubated for 12 h, and treated as above. For ethidium bromide treatment, BHI broth cultures of selected prt^+, $haem^+$ colonies were inoculated into broth containing 50, 75, and 100 μg ethidium bromide·mL^{-1} (Sigma Chemical Co., St. Louis, MO) (approximately 10^5 cells·mL^{-1} final concentration), incubated for 30 h, and plated as above.

Spontaneous rifampicin resistant mutants were isolated by plating approximately 5×10^8 cells onto BHI agar plates containing 100 μg rifampicin·mL^{-1} (Sigma Chemical Co., St. Louis, MO). Rifampicin resistance was used in initial experiments to ensure the purity of cultures when low frequencies of prt^-, $haem^-$ variants were isolated. Strains used for virulence studies (Table 3) were not selected for rifampicin resistance.

Biochemical Methods

Plasmids were isolated using the crude cell lysate procedure of Meyers et al. (1976) and a modification of the procedure of Kado and Liu (1981). In this modification, the cell lysate was incubated at 55°C for 1 h and extracted with 2 volumes of phenol–chloroform solution (1:1 v/v) three times. The aqueous phase was brought to 0.3 mol sodium acetate·L^{-1} and 2 volumes of 95% ethanol (−20°C) were added. Samples were held at −20°C for 12 h to precipitate the DNA. The plasmid DNA was collected by centrifugation at 12 000 × *g* at −10°C for 20 min and the precipitate was resuspended in 50 μL of E buffer (Kado and Liu 1981). Following the addition of 4 μL of dye mix (Kado and Liu 1981) to 10 μL of plasmid sample, the samples were run on a horizontal gel electrophoresis apparatus (Life Sciences Design and Development Facilities, UCLA) for 12 h at 100 V, 50 mA, using 0.35, 0.7, or 1.0% agarose (Seakem, FMC Corp., Marine Colloids Div., Rockland, ME) gels. The gels were stained with 1 μg ethidium bromide·mL^{-1} and destained for 30 min in H_2O.

To determine molecular masses, covalently closed circular forms of the plasmids were isolated by the modified method of Kado and Liu (1981) described above, except that the final phenol–chloroform-extracted aqueous phase was dialyzed (4.5-nm pore diameter dialysis tubing) against E buffer for 12 h at 4°C for phenol–chloroform removal. The precipitated plasmid samples were resuspended in 50 μL of E buffer containing 1 mmol $MgSO_4 \cdot L^{-1}$. Deoxyribonuclease (DNAse) (specific activity 3000 units·mg $protein^{-1}$) (Miles Laboratories Ltd., Rexdale, Ont.) was added (1 μL) to 10-μL samples of the resuspended plasmid preparations (containing approximately 5 μg of DNA) to give final concentrations of 0.03–3 units DNAse·mL^{-1}. The samples were incubated at 37°C for 15 min. Ethylenediaminetetraacetic acid (disodium salt) was added to a final concentration of 20 mmol·L^{-1} and the samples were stored on ice for 15 min. Dye mix (Kado and Liu 1981) was then added and the resulting 16-μL samples were immediately subjected to agarose gel (1.2%) electrophoresis as described above using λ Hind III digest DNA (Bethesda Research Laboratories, Inc., Rockville, MD.) as a standard.

Copy number determinations were made on the series of four small plasmids in the 3.5–4.0 megadalton (Md) range in *A. salmonicida* Rest. 80204. Following lysis of 2.5×10^{10} cells (Meyers et al. 1976), 5 μg of λ Hind III digest DNA was added. After ethanol precipitation, the plasmid–λ Hind III digest mixture was resuspended in 100 μL of E buffer and 20-μL aliquots were electrophoresed on 1% agarose gel. To provide absolute standards, 0.5, 0.2, 0.1, and 0.05 μg of λ Hind III digest DNA were run simultaneously in adjacent tracks. The gels were stained with ethidium bromide and photographed at several exposures on Pan-X film (Kodak) with a red (25A) filter. A positive was printed on Kodak (10.2 × 12.7 cm) Professional Copy Film (No. 4125), and the band intensities were recorded on a Hoefer gel scanner (model GS300). Copy numbers were determined following integration of peak areas. First, the absolute peak area per microgram of DNA was calculated using the standards in the control tracks (standard deviation was

TABLE 1. *Aeromonas salmonicida* strains and sources.

Strain	Source
ATCC 14174	American Type Culture Collection
Mac 79-38R	Fish Health Unit, Department of Fisheries and Oceans, Halifax, N.S.
Rest. 80204, SS70, AS1, AS Kejimkjuik	Connaught Research Institute, Toronto, Ont.

TABLE 2. Loss of extracellular protease and haemolysin activities from *A. salmonicida* (nt = not tested).

Strain	% prt⁻, haem⁻ colony-forming units (cfu)		
	From one subculture of lyophilized culture[a]	From subculture of selected prt⁺, haem⁺ colonies[b]	Following incubation of prt⁺, haem⁺ subcultures in the presence of ethidium bromide (50 $\mu g \cdot mL^{-1}$) for 30 h
ATCC 14174	nt	0.1–0.4	0.5–3.0
Mac. 79-38R	nt	<0.1	0.3–2.0
Rest. 80204	<0.1–97.0	<0.1	0.7–15.0
SS70	<0.1	<0.1	17.0–18.0
AS1	6.0–40.0	nt	nt

[a]Each strain tested was subcultured from two to five vials of lyophilized culture, and 2000–5000 colonies were examined.

[b]Results are from two to six separate experiments. From each subculture, 2000–5000 colonies were examined.

±21%). Next, the total amount of each plasmid was determined by combining the open and closed circular peak areas, correcting for the decreased absorbance of the closed circular forms as described by Projan et al. (1983). Finally, the copy number was obtained by correcting for the average loss observed for the six largest coextracted λ Hind III digest fragments.

To observe the A-layer, bacteria from 12-h-old BHI broth cultures were washed three times in distilled water and collected each time by centrifugation at 4°C and 4360 × *g* for 10 min. The cells were resuspended in distilled water and appropriate dilutions were negatively stained with 1% uranyl acetate. The stained cells were examined with a Philips EM400T electron microscope by transmission electron microscopy.

Biochemical tests (Table 4) were done according to the method of Blair et al. (1970).

Virulence Studies

Bacterial challenge studies were done on fish by a bath immersion method. Challenge cultures were grown in BHI broth to an OD at 590 nm of approximately 1.0. Brook trout (*Salvelinus fontinalis*), weighing approximately 8 g each, were challenged by immersion for 2 min at 18°C in serial 10-fold dilutions (saline) of the test strain and returned to the tank. Fish were monitored for mortalities twice daily over a period of 14 d. *Aeromonas salmonicida* was detected in all mortalities by streaking a kidney sample on skim milk overlay medium. At the end of the experimental period, survivors of the highest challenge dose were similarly checked without detecting *A. salmonicida*.

Results and Discussion

Isolation of Variants Lacking Extracellular Protease and Haemolysin Activities

Strains of *A. salmonicida* were tested for the loss of extracellular protease and haemolysin on skim milk agar and blood agar medium, respectively. Sufficient colonies were examined to detect the loss of these activities at a frequency of ≥0.1%. Colonies producing minimal or undetectable zones of clearing after incubation at 22°C for 72 h were considered prt⁻, haem⁻. Colonies showing zones of casein hydrolysis less than the wild-type but greater than the prt⁻ variants produced a mixed culture of prt⁺ and prt⁻ colonies after subculturing in BHI broth. We observed a high frequency of spontaneous loss from one strain (0.1–0.4%) and a high frequency of induced loss from four strains (0.3–18%) treated with ethidium bromide, a plasmid curing agent (Table 2). Subcultures of some lyophilized cultures of two strains examined also contained a high percentage of prt⁻, haem⁻ variants. All prt⁻ variants isolated were also haem⁻ and similarly all haem⁻ variants isolated were prt⁻. We do not know the reason for the apparent coordinated loss of these two extracellular activities.

Because of the high frequency of prt⁻, haem⁻ variants isolated from cultures treated with ethidium bromide and the inhibitory effect of this compound on the growth of the organisms, the frequency of resistance to ethidium bromide ($75\ \mu g \cdot mL^{-1}$) was examined and appeared to occur at a normal mutation rate (0.5×10^{-7} to 6.7×10^{-7}). The number of ethidium bromide resistant mutants that lacked protease activity was high (13–35%). However, in prt⁻, haem⁻ variants that were not isolated as resistant to ethidium bromide, we did not observe a direct relationship between the loss of protease activity and the acquisition of ethidium bromide resistance, and high frequencies of prt⁻ variants were isolated from some strains that were not exposed to ethidium bromide.

Plasmid Analyses

Plasmid analyses (Kado and Liu 1981) were carried out to determine if the high frequency of loss of extracellular protease and haemolysin from *A. salmonicida* was correlated with the curing of a plasmid. Except for the atypical Kejimkjuik isolate (Paterson et al. 1980b), the wild-type strains carry four similar small plasmid species (Fig. 1) with molecular masses estimated from the linear plasmid forms to be 3.5, 3.7, 3.8, and 4.0 Md (Fig. 2B). Similar plasmid profiles for other *A. salmonicida* strains have been reported by Toranzo et al. (1983), who estimated molecular masses of 3.5–4.2 Md for the four plasmid species. In addition, the ATCC 14174 (Fig. 1A, lane c), Rest. 80204 (Fig. 1D, lane a), and SS70 (Fig. 1D, lane b) wild-type strains each carry a larger plasmid. The Kejimkjuik strain carries three larger plasmids but none of the four smaller plasmids (Fig. 1C, lane c), a result that tends to confirm its atypical nature.

A second plasmid isolation procedure (Meyers et al. 1976) failed to reveal the larger plasmids on the gels and produced apparent multiple forms of the four smaller plasmids, due to the retention of nicked plasmids during isolation (Fig. 2A). The apparent relationship between the multiple forms of the four

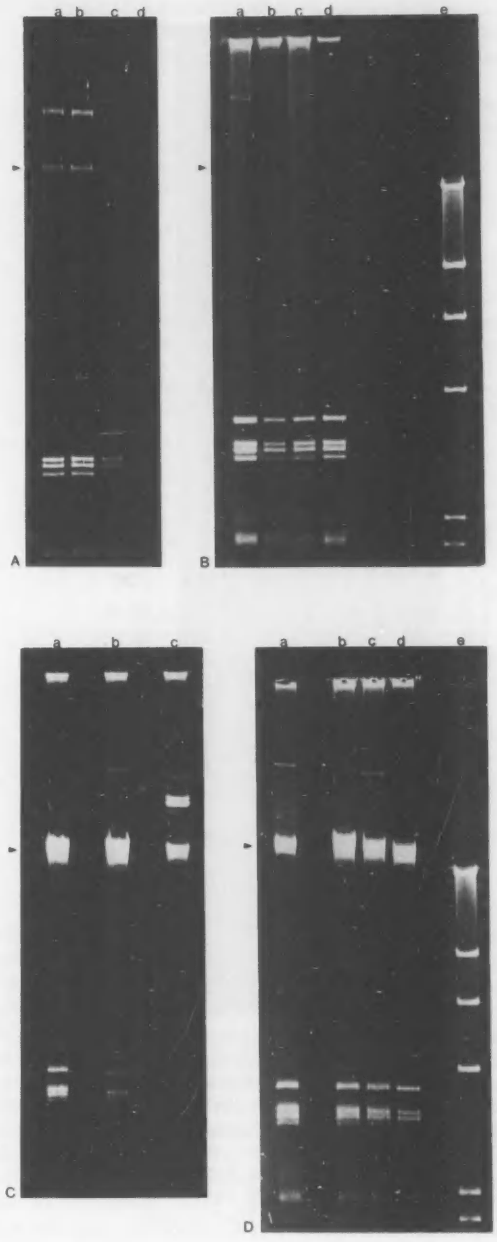

FIG. 1. Agarose gel electrophoresis of plasmid DNA from strains of *A. salmonicida* isolated using the method of Kado and Liu (1981). (A) 1.0% agarose gel: lane a, ATCC 14174.1 (prt^+, $haem^+$); lane b, ATCC 14174.2 (prt^-, $haem^-$); lane c, ATCC 14174 (wt); lane d, ATCC 14174.3 (prt^-, $haem^-$). (B) 1.0% agarose gel: lane a, Rest. 80204 (wt); lane b, Rest. 80204.1 (prt^-, $haem^-$); lane c, Mac. 79-38R (wt); lane d, Mac. 79-38R.1 (prt^-, $haem^-$); lane e, λ Hind III digest. (C) 0.7% agarose gel: lane a, Rest. 80204 (wt); lane b, Rest. 80204.2 (prt^-, $haem^-$); lane c, AS Kejimkjuik (wt). (D) 1.0% agarose gel: lane a, Rest. 80204 (wt); lane b, SS70 (wt); lane c, SS70.1 (prt^+, $haem^+$); lane d, SS70.2 (prt^-, $haem^-$); lane e, λ Hind III digest. Arrowheads denote linear chromosomal DNA.

small plasmids was confirmed by limited DNAse digestion of the covalently closed circular forms of these plasmids (Fig. 2B). Copy numbers for the four small plasmids, using the Rest. 80204 strain, were estimated to be 57, 57, 55, and 35 copies per cell in descending order of size, values similar to those reported for smaller plasmid species by Projan et al. (1983). We estimate the limit of detection with the methods used to be five copies per cell for plasmids in this size range.

Regardless of the plasmid isolation method used, plasmid profiles of the prt^-, $haem^-$ variants from the four wild-type strains failed to show any loss of a plasmid correlated with the loss of the extracellular enzyme activities. Although *A. salmonicida* Rest. 80204.1 (prt^-, $haem^-$ variant of the wild-type Rest. 80204) did not possess the larger plasmid (Fig. 1B, lane b), other prt^-, $haem^-$ variants of Rest. 80204 retained this plasmid (Fig. 1C, lane b). Also, prt^+, $haem^+$ (Fig. 1A, lane a) and prt^-, $haem^-$ (Fig. 1A, lane b) variants of ATCC 14174 were isolated that had lost the 4.0-Md plasmid. Therefore, while two cases of plasmid curing were observed, these were not associated with the loss of the extracellular enzyme activities.

Our inability to detect changes in the plasmid profiles between the wild-type strains and their prt^-, $haem^-$ variants does not rule out the possibility that these extracellular activities are plasmid-coded products or that another plasmid-coded product may be involved, perhaps in the release of these activities from the cells (Howard and Buckley 1983). The plasmid isolation methods used may have been inadequate to allow detection of particular plasmids, especially if they were present at less than five copies per cell (Roberts and Smith 1980). Also, the genes may reside, in an integrated form, on a lysogenic phage or episome, and excision and subsequent loss of these elements from the chromosome would not be detected by our methods (Barksdale et al. 1961; Zabriskie 1964). It might be noted that other abrupt losses of A-layer (Ishiguro et al. 1981) and of sulfadiazine resistance (Toranzo et al. 1983) from *A. salmonicida* could not be correlated with the loss of detectable plasmids.

Virulence Studies

The results of Sakai (1977) and Cipriano et al. (1981) indicated that an extracellular protease is a major pathogenic element produced by *A. salmonicida*. The role of extracellular protease and haemolysin in the virulence of the organism was examined using two virulent strains, Rest. 80204 and SS70, and their prt^-, $haem^-$ derivatives, in a more natural host–pathogen system. The lethal dose (LD) values were based on bath immersion studies. The LD_{100} for wild-type SS70 was approximately 10^4 colony-forming units (cfu)·mL^{-1} and for wild-type Rest. 80204 was approximately $\leq 10^6$ cfu·mL^{-1} (Table 3).

Challenging brook trout (*S. fontinalis*) with Rest. 80204.2 (a spontaneously occurring prt^-, $haem^-$ variant of Rest. 80204) did not result in a significant change in the LD_{100} from that observed with the parent strain (Table 3). Also, there was no apparent selection for the prt^+, $haem^+$ phenotype when bacteria were reisolated from dead fish that had been challenged with a mixed culture of the prt^+, $haem^+$ wild-type strain and its prt^-, $haem^-$ derivative (data not shown). Challenge cultures prepared from a prt^+, $haem^+$ and a prt^-, $haem^-$ (Rest. 80204.3) colony, isolated after treatment of wild-type Rest. 80204 cells with ethidium bromide, showed similar LD_{100} values to the wild-type strain. Thus, in this strain, a loss of detectable extracellular protease and haemolysin (spontaneously or following ethidium bromide treatment) was not correlated with a loss of virulence.

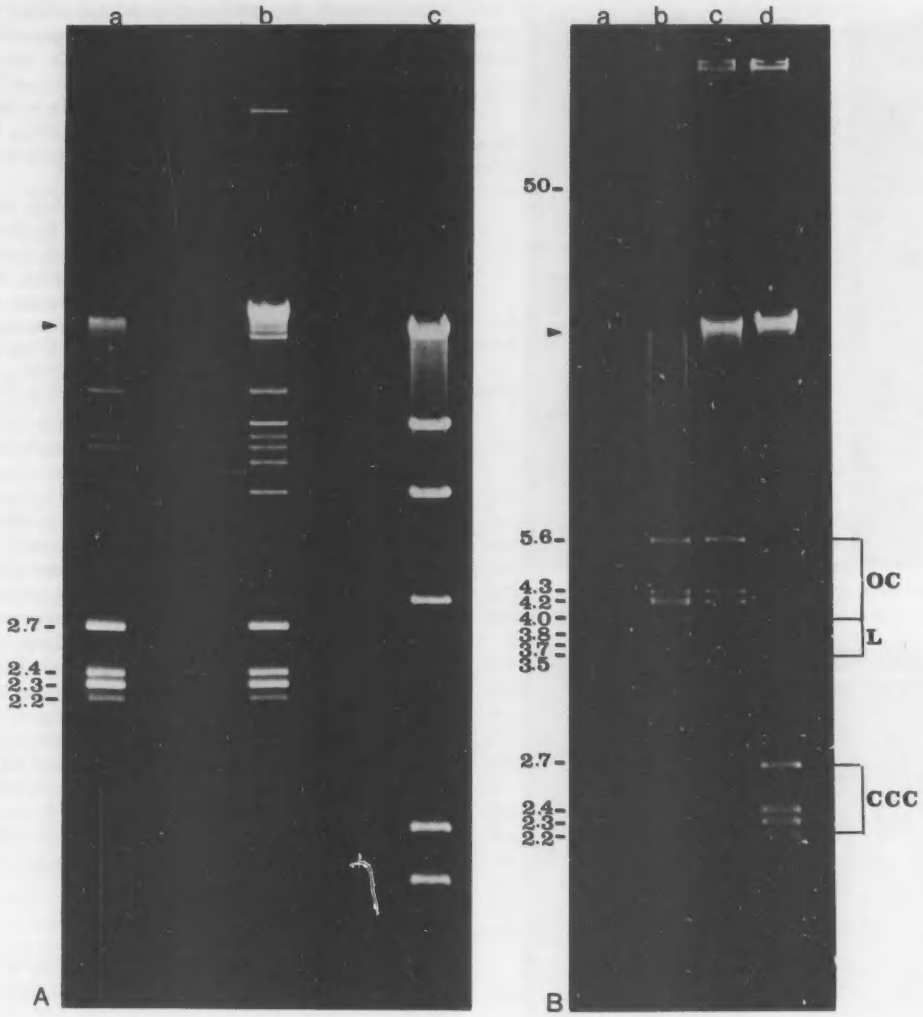

FIG. 2. Comparison of plasmid profiles by agarose gel electrophoresis using two different methods for plasmid isolation from *A. salmonicida*. (A) From the method of Meyers et al. (1976) (1.0% agarose gel): lane a, Rest. 80204; lane b, Rest. 80204 plus λ Hind III digest; lane c, λ Hind III digest. (B) From the method of Kado and Liu (1981), and the effect of limited DNAse digestion on the plasmid profiles obtained with this method (1.2% agarose gel). Rest. 80204 treated with DNAse for 15 min: lane a, 3.0 units·mL^{-1}; lane b, 0.3 unit·mL^{-1}; lane c, 0.03 unit·mL^{-1}; lane d, no DNAse digestion. Arrowheads, linear chromosomal DNA; CCC, covalently closed circular plasmids; OC, open circular plasmids; L, linear plasmids. Sizes are in megadaltons and are based on extrapolation from linear λ Hind III digest fragments.

In contrast with Rest. 80204, both prt$^+$, haem$^+$ (SS70.1) and prt$^-$, haem$^-$ (SS70.2) colonies, isolated from wild-type SS70 cells treated with ethidium bromide, were avirulent (LD_{50} increased to $>10^7$ cfu·mL^{-1}). No spontaneous prt$^-$, haem$^-$ variants of SS70 were obtained. The reason for this difference between the strains is unknown. The prt$^-$, haem$^-$ (SS70.2) variant was reisolated from four dead fish (Table 3) and when used to challenge fish did not demonstrate any increase in virulence (data not shown). Plasmid profiles of virulent wild-type SS70 and its avirulent derivatives were similar except for a deletion in the large plasmid from SS70.1 (Fig. 2D, lanes b–d). This deletion did not appear to be related to the loss of virulence, since it was not detected in the plasmid profile from avirulent SS70.2. Therefore, the decreased virulence of SS70.1 (prt$^+$) and SS70.2 (prt$^-$) was not correlated with the loss of protease and haemolysin activities or the detectable loss of a plasmid.

Biochemical Tests

Paterson et al. (1980a) reported that results of biochemical

TABLE 3. Comparison of virulence of two wild-type strains of *A. salmonicida*, and strains isolated after loss of protease and haemolysin activities or ethidium bromide treatment.

Strain	Treatment	A-layer	Protease	Haemolysin	No. fish challenged	No. of mortalities	Lethal dose (LD) ($cfu \cdot mL^{-1}$)
SS70 (wt)	–	+	+	Weak	20	20	$LD_{100} \sim 10^4$
SS70.1 (2 tests)	EB.50[a]	+	+	Weak	20, 20	0, 0	$LD_{50} > 10^{7b}$
SS70.2 (2 tests)	EB.50	+	–	–	20, 20	1, 3	$LD_{50} > 10^7$
Rest. 80204	–	+	+	+	22	22	$LD_{100} \leq 10^{6c}$
Rest. 80204.2[d]	–	+	–	–	22	22	$LD_{100} \leq 10^6$
Rest. 80204	EB.50	+	+	+	24	24	$LD_{100} \leq 10^6$
Rest. 80204.3	EB.50	+	–	–	22	22	$LD_{100} \leq 10^6$

[a]Wild-type subcultures were treated with ethidium bromide ($50\ \mu g \cdot mL^{-1}$) for 30 h, and clones (prt^+, $haem^+$ or prt^-, $haem^-$) were subsequently selected after plating on skim milk overlay plates.
[b]No significant mortalities at highest challenge dose ($10^7\ cfu \cdot mL^{-1}$).
[c]100% mortality at lowest challenge dose ($10^6\ cfu \cdot mL^{-1}$) tested using Rest. 80204.
[d]Spontaneously occurring prt^-, $haem^-$ isolate from Rest. 80204.

TABLE 4. Some biochemical test reactions for the *A. salmonicida* strains examined. All wild-type strains and variants examined gave similar results for the other tests performed (positive for lysine decarboxylase and fermentation of glucose, fructose, mannose, and mannitol; negative for indole production).

Strain	Selection for rifampicin resistance	Casein hydrolysis	Haemolysin activity	Gelatin hydrolysis	A-layer	Fermentation	
						Galactose	Sucrose
SS70 (wt)	–	+	Weak	+	+	Weak	Weak
Rest. 80204 (wt)	–	+	+	+	+	+	Weak
Mac 79-38R (wt)	–	+	+	+	+	+	AG[a]
ATCC 14174 (wt)	–	+	Weak	+	–	+	Weak
ATCC 14174.4	+	+	Weak	+	–	+	Weak
ATCC 14174.5-.9[b]	+	–	–	v[c]	–	+	Weak

[a]Acid and gas produced.
[b]Clones selected as prt^- on casein overlay agar plates following treatment of ATCC 14174.4 cells with ethidium bromide.
[c]Variable.

tests for several *A. salmonicida* strains showed variation within the species. We also found some species variation between the four typical wild-type strains examined (Table 4). However, all variants tested showed similar reactions to their respective parent wild-type strains except for the loss of extracellular protease and haemolysin activities. The one exception was variants of *A. salmonicida* ATCC 14174, which were initially selected as rifampicin resistant (ATCC 14174.4) and subsequently isolated as prt^-, $haem^-$ clones following ethidium bromide treatment (ATCC 14174.5–.9). Some (two of five tested) did not hydrolyze gelatin on gelatin overlay agar plates (Table 4). However, these variants had plasmid profiles similar to the parent wild-type ATCC 14174 strain. *Aeromonas salmonicida* produces at least two separate extracellular proteolytic activities (Sheeran and Smith 1981): (1) a serine protease characterized by Shieh and McLean (1975) and implicated in pathogenicity which hydrolyzes casein, gelatin, and collagen and (2) a gelatinase showing activity against gelatin and collagen but not hydrolyzing casein.

The A-layer has been shown to be necessary for the virulence of *A. salmonicida* (Udey and Fryer 1978; Ishiguro et al. 1981). All strains used for virulence testing possessed an A-layer as observed by transmission electron microscopy. However, some strains that possess an A-layer are avirulent (Udey 1978) as in the case of the avirulent variants of SS70 tested here.

Because of the pathological effects observed following the injection of *A. salmonicida* extracellular products into fish and the implication of extracellular protease as a major pathogenic factor (for partial review see Cipriano et al. 1981), the results obtained here are significant. Attenuation of the SS70 strain occurred without coincident detected loss of the A-layer, plasmids, or extracellular protease and haemolysin activities. Thus, the virulence of *A. salmonicida*, at least in part, is due to other unknown bacterial factors. Virulence studies with Rest. 80204 and its prt^-, $haem^-$ variants lend further support to these observations, since, in this strain, the loss of protease and haemolysin activities was not correlated with a loss of virulence. Virulence, at least in the acute form of furunculosis, appears to be independent of the two extracellular enzyme activities. Thus, in the peracute or acute forms of furunculosis, it is unknown whether death results from organ dysfunction caused simply by bacterial growth or by some unidentified extracellular bacterial product(s).

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Statistical Inference in Life-Table Experiments: The Finite Rate of Increase

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Life-table experiments are frequently used to examine the effects of food level, toxicants, and other experimental treatments on a population's finite rate of increase λ. Although methods for computing the variance of λ have been suggested, the sampling distribution of λ, which is needed for statistical inference, has not been described. We used Monte Carlo procedures to simulate sampling distributions of λ for a variety of assumptions regarding survivorship and fecundity schedules and initial cohort sizes. The distribution of λ can be bimodal when cohort size is small and when juvenile mortality is large. Under these circumstances the probability that none of the initial cohort members reproduces is high enough to produce a significant frequency of zero values for λ. Zero therefore becomes the lower mode of the distribution. Many commonly observed mortality schedules and commonly used cohort sizes yield distributions of λ that are skewed toward low values. Although the skewness and variance of distributions of λ decrease as cohort size increases, these distributions are asymptotically non-normal. Normal-based statistical procedures for comparing experimental estimates of λ may therefore be misleading. To compare estimates of λ obtained from life-table experiments, we recommend using a Monte Carlo approach to generate sampling distributions of λ and then comparing these distributions directly. We illustrate this procedure with life-table data from *Daphnia pulex* cohorts raised at different levels of pH. We also show that a Taylor series variance estimator yields confidence intervals for λ that approximate those obtained from simulations. This variance estimator is less conservative, and therefore more useful, than a previous estimator derived by Lenski and Service (1982. Ecology 63: 655–662).

On utilise souvent des expériences relatives aux tables de survie pour étudier l'incidence de la quantité d'aliments, de substances toxiques et d'autres traitements expérimentaux sur le taux fini d'accroissement (λ) d'une population. Quoiqu'on ait avancé des méthodes pour le calcul de la variance de λ, sa distribution dans l'échantillon, nécessaire à la déduction statistique, n'a pas été décrite. Les auteurs utilisent les procédures de Monte Carlo pour simuler les distributions de λ dans un échantillon en fonction de diverses hypothèses sur les régimes de survie et de fécondité et sur les tailles initiales des cohortes. La distribution de λ peut être bimodale quand la taille de la cohorte est petite et que la motalité des juvéniles est élevée. Dans ces circonstances, la probabilité qu'aucun des membres de la cohorte initiale ne se reproduise est assez élevée pour donner lieu à une fréquence significative de valeurs nulles pour λ. Zéro devient donc le mode inférieur de la distribution. Plusieurs régimes de mortalité fréquemment observés et un grand nombre de tailles de cohorte utilisées génèrent des distributions de λ qui sont désaxées vers la gauche. Quoique l'asymétrie et la variance des distributions de λ diminuent quand la taille de la cohorte augmente, ces distributions ne sont pas normales par rapport à l'asymptote. Les procédures statistiques normales pour la comparaison d'estimations expérimentales de λ sont donc peut-être trompeuses. Afin de comparer des estimations de λ obtenues d'expériences portant sur les tables de survie, les auteurs recommandent l'utilisation des procédures de Monte Carlo pour l'obtention de distributions de λ dans un échantillon, qui sont ensuite comparées directement. On illustre la procédure à l'aide de données sur la survie de cohortes de *Daphnia pulex* élevées à différents pH. On montre aussi qu'un estimateur de la variance des séries Taylor génère des intervalles de confiance de λ qui s'approchent de ceux obtenus par simultation. Cet estimateur de la variance est moins modeste, et donc moins utile, qu'un estimateur précédent, calculé par Lenski et Service (1982. Ecology 63: 655–662).

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The methodology of a life-table experiment is straightforward. Individual animals are isolated at birth and raised under a defined set of experimental conditions, and the ages at which they reproduce and die are recorded. Lotka (1907a, 1907b) was the first to demonstrate how age schedules of fecundity and mortality measured in this way could be used to calculate fundamental parameters of population growth. He showed, for example, that a population's per capita growth rate r could be calculated from

$$(1) \qquad 1 = \int_0^\infty l(x)m(x)\, e^{-rx}dx$$

in which $l(x)$ = the proportion of animals that survive to age x and $m(x)$ = the mean number of offspring produced in a unit of time by animals of age x. In practice, the population's finite rate of increase λ, which equals $e^{r\Delta t}$ (Leslie 1945), is calculated by using a finite form of Eq. 1 (Michod and Anderson 1980) because discrete time intervals of length Δt are used in place of continuous observations to determine time-averaged, age-specific values of fecundity and survivorship. The intrinsic rate of increase r is then calculated from λ.

Life-table experiments are frequently used by zooplankton ecologists to study nutrition (Ingle et al. 1937; Frank et al. 1957; Arnold 1971; Porter and Orcutt 1980; Robertson and Salt 1981), aging (King 1967; King and Miracle 1980), population dynamics (Halbach and Halbach-Keup 1974; Goulden et al. 1982b; Wyngaard 1983), effects of temperature variation (Orcutt and Porter 1983), and toxicology (Winner and Farrell 1976; Daniels and Allan 1981; Gentile et al. 1982; Walton et al. 1982). In addition, this type of experiment is used by evolutionary ecologists because the finite rate of increase is frequently defined as a measure of a population's fitness (Roughgarden 1979).

Despite the widespread applicability of life-table experiments and the evolutionary significance of λ, the statistical properties of λ and other life-table parameters have received only limited attention (Keyfitz 1977; Lenski and Service 1982). Theoretical studies have concentrated on examining how stochastic variations in age-specific survivorship and fecundity influence projections of population size and age structure through time. Pollard (1966) and Sykes (1969), for example, provided closed-form expressions for the mean and variance of the age distribution of population projections. These authors showed that the expected value of such projections is equivalent to the age distribution predicted by Leslie's (1945) deterministic model. Boyce (1977) and Daley (1979) recently examined the behavior of expected values of r and λ for stochastically projected populations. Their results initiated some debate about how to calculate these growth rates for populations that experience long-term variations in age-specific fecundity and survivorship (Cohen 1979; Tuljapurkar and Orzack 1980; Slade and Levenson 1982). Although important to furthering demographic theory, these studies offer limited help to ecologists who want to use life-table experiments to estimate and make inferences about parameters such as λ. Furthermore, if life-table tests are to provide useful information on the sublethal effects of toxicants, then it is essential that the statistical properties of parameters such as λ be known.

With the exception of Lenski and Service (1982), none of the above-mentioned studies addressed how random errors produced by experimental estimation of age-specific fecundity and mortality schedules affect the sampling distributions of life-table parameters. This lack of theoretical development has forced empiricists to either ignore the statistical properties of λ (e.g. Porter and Orcutt 1980; Daniels and Allan 1981; Walton et al. 1982) or to compute a mean and variance using values of λ computed for each individual of the cohort (Hebert et al. 1982). The former approach ignores valuable information while the latter approach yields biased estimates of the mean and variance of λ (Lenski and Service 1982). In addition, statistical inferences that are based on assumptions of normality for these biased means may be seriously incorrect because λ is an implicit nonlinear function of $l(x)$ and $m(x)$ and there is no a priori reason to assume that the sampling distribution approximates a normal-based sampling distribution such as the t-distribution.

In this paper we illustrate the importance of examining the underlying distribution of λ, the population's finite rate of increase. Using Monte Carlo simulation, we generate sampling distributions of λ for populations of zooplankton experiencing hypothetical, but realistic, fecundity and mortality schedules. These results demonstrate the effects of cohort size and age-specific patterns of survivorship and fecundity on the sampling distribution of λ. Next we obtain the sampling distribution of λ from empirical values of $l(x)$ and $m(x)$ and demonstrate how this distribution may be used in hypothesis testing. We conclude by comparing our approach with other variance estimators of λ and by showing how our approach can also be used to help design experiments.

Methods

Each set of observations from a life-table experiment can be considered as a single realization of complex, underlying, stochastic processes governing survival and reproduction. Thus the finite rate of increase is a function of a set of random variables and is therefore itself a random variable (Mood et al. 1974). Due to the complexity of the underlying processes, the nonlinearity of Eq. 1, and the lack of an explicit solution to Eq. 1, it is not possible to derive a closed-form expression for the distribution of λ. The probability density function (pdf) of λ is the totality of all possible outcomes of the underlying stochastic processes. The pdf of λ can therefore be approximated by sampling these processes an arbitrary, but large, number of times. The resulting approximate pdf serves as the null distribution and provides a basis for statistical inference regarding experiments.

In our simulations we calculate λ using the following discrete equivalent of Eq. 1:

$$(2) \qquad 1 = \sum_{x=1}^{A} \lambda^{-x} l_x m_x$$

in which l_x = the probability of survival to age class x, m_x = the mean number of offspring born to an individual in age class x, and A = the maximum number of age classes. We estimate the statistical properties of λ using Monte Carlo simulations by assuming that l_x and m_x, $x = 1, 2, \ldots, A$, are random vectors drawn from specified underlying distributions (see submodels below).

To be useful, a Monte Carlo model must be firmly grounded in statistical theory and yet closely tied to key biological characteristics. Our model is based on characteristics of parthenogenetic crustaceans (parameters in Table 1) but could easily be extended to other species. Submodels are used to generate sample survivorship and fecundity curves that are

TABLE 1. Parameter values used in simulations. Values for N_0, c, b, and k were varied among simulations.

Parameter		Simulation value
A	= maximum number of age classes	48
Δa	= duration of each age class	1 d
I_{max}	= total number of iterations	1000
N_0	= initial cohort size	5, 10, 20, 40, 1000 individuals
c	= Weibull shape parameter	1.0, 2.5, 5.0
b	= Weibull scale parameter	Adjusted so that on average 1% of the cohort survives to be $A\Delta a$ days old
x_r	= age class at birth of first offspring	10
α	= type A clutch size parameter	5.0756×10^{-6}
β	= type A clutch size parameter	4.1597
μ_{max}	= type B clutch size parameter	20
γ	= type B clutch size parameter	3.51599×10^{-2}
θ	= type C clutch size parameter	39.719
ψ	= type C clutch size parameter	2.9224×10^{-1}
C	= integral reproductive output	465 offspring·female^{-1}
h	= number of age classes that comprise one egg developmental period	3
k	= fractional coefficient of variation of clutch size	0.15, 0.30, 0.60

representative of those commonly observed in life-table experiments with zooplankton.

Mortality Submodel

In the mortality submodel a two-parameter Weibull distribution is used to describe typically observed survivorship patterns. The Weibull distribution is widely used as a model of lifetimes of materials, particularly in industrial applications (Johnson and Kotz 1970). Recently, Pinder et al. (1978) advocated the Weibull model for natural populations and demonstrated that the Weibull distribution can closely mimic the Type I through Type III survivorship patterns described by Deevey (1947). Thus the Weibull model satisfies criteria established by Keyfitz (1982) for effective demographic forecasting and modeling.

The Weibull distribution can be formulated (Somers et al. 1980) such that l_x is a function of age class x, the duration of each age class Δa, a shape parameter c, and a scale parameter b:

$$l_x = \exp\,[-(x\Delta a/b)^c]. \tag{3}$$

The probability that animals aged $(x-1)\cdot\Delta a$ will survive to reach the next age class x can be written as

$$p_x = l_x/l_{x-1} \tag{4}$$

or, substituting Eq. 3 into 4:

$$p_x = \exp\,\{[(x-1)\Delta a/b]^c - (x\Delta a/b)^c\}. \tag{5}$$

Thus the Weibull model can be used to generate age-specific probabilities of survival over all age classes.

We used these p_x values to generate random survivorship curves for sample cohorts as follows. In a sample cohort initially comprising N_0 animals, we set S_j to be the outcome of a random process such that $S_j = 0$ when individual j dies and $S_j = 1$ when it lives through the age interval Δa. Since p_x describes the probability of surviving, S_j is a Bernoulli-distributed random variable with parameter p_x. An individual from age class $x-1$ will survive to age class x with probability $Pr(S_j = 1) = p_x$. Since the probability of survival is independent of density, the number of cohort members surviving the interval Δa is a function of the outcomes of N_x independent Bernoulli trials. Thus a recursive relation between N_x, the number of animals from age class x, and N_{x+1}, the number that survive to reach the next age class, can be written as

$$N_{x+1} = \sum_{j=1}^{N_x} S_j. \tag{6}$$

In the model, values of S_j are determined by comparing a uniformly distributed random variable U_j with range [0, 1] to p_x. If $U_j < p_x$, then $S_j = 1$; otherwise, $S_j = 0$.

From statistical theory, the sum of independently and identically distributed Bernoulli random variables is itself a binomially distributed random variable with parameters N_x and p_x (Mood et al. 1974). In the present formulation, however, N_x is also a random variable because it depends on the outcomes of previous sequential Bernoulli trials over the age-class interval $[1, x-1]$. This formulation is known as a chain binomial model (Chiang 1968; Pielou 1977), and it can be shown that the pdf of cohort size at each age class x is given by

$$f(N_x) = l_x^{N_x}(1 - l_x)^{N_0 - N_x}, \quad N_x \in \{0, 1, \ldots, N_0\} \tag{7}$$

where N_0 is the original size of the cohort. From Eq. 7 it is evident that all cohort sizes from zero to N_0 have a finite probability of occurrence at any age class x. The probability of large N_x (near N_0), of course, becomes very small as l_x approaches zero. Moreover, since N_x depends on random events occurring over the age-class interval $[1, x-1]$, the realized trajectory of cohort sizes is always monotonically nonincreasing.

We summarize our procedure for computing a realized schedule of age-specific survivorship, which we denote as $\hat{l}_x$, as follows. First, we hypothesize a particular form for the survivorship curve by selecting parameters c and b of a Weibull distribution function (Eq. 3). Age-specific probabilities of survival are then computed by using Eq. 5. For each realization these probabilities serve as parameters for sequentially applied Bernoulli trials at each age class. The number of Bernoulli trials at a given age class, N_x, determines the number alive in the next age class (Eq. 6). This sequence results in a distribution of N_x values that follow a chain binomial model. The realized l_x curve is then computed from this distribution by

$$\hat{l}_x = N_x/N_0. \tag{8}$$

In this way the realized l_x values ($\hat{l}_x$) are closely tied to observable patterns of survival and a particular cohort size. Unlike other stochastic age-structured models (Daley 1979; O'Neill et al. 1981), the realized l_x curve can only assume values that are integer multiples of $1/N_0$. We believe that this procedure more realistically reflects the outcomes that are possible when a cohort of newborn animals is followed through time.

For the simulation study of hypothetical populations, we parameterized the Weibull model by assuming that the maximum age was 48 d ($A = 48$, $\Delta a = 1$ d) and that 1% of the initial cohort of N_0 animals survived to reach this age. In this way, setting a value for the shape parameter c determines the value of b (Eq. 3). We varied c from 1.0 to 2.5 to 5.0 to yield three survivorship schedules (Fig. 1).

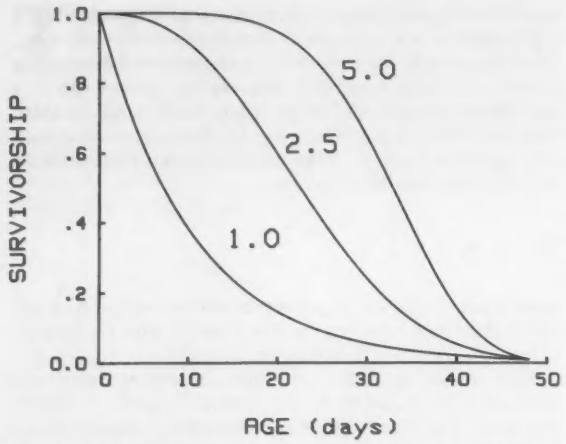

FIG. 1. Weibull distribution curves used to model survivorship. Each curve is identified by a number that corresponds to the value of the Weibull shape parameter c.

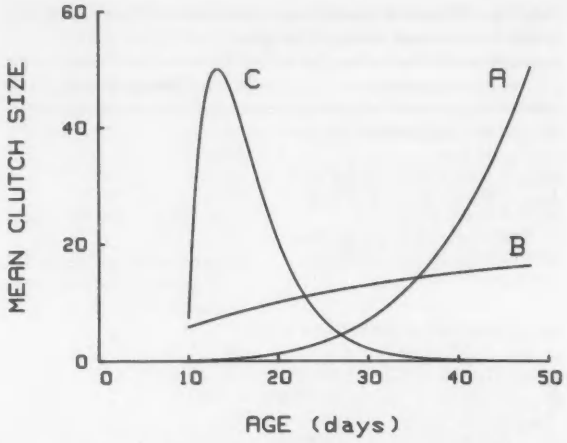

FIG. 2. Mean clutch size was predicted by three types of reproductive strategies: A, B, and C (which correspond to Eq. 10, 11, and 12, respectively).

Reproduction Submodel

We modeled reproduction by assuming that the mean number of offspring produced by animals in age class x, (m_x), is the product of two random variables: the average clutch size of females in age class x, (F_x) and the fraction of those females that hatch offspring (f_x). This formulation allows us to account for variation in m_x that arises from variability in both the magnitude and schedule of reproductive events. While F_x determines the average number of offspring produced by fecund females, f_x accounts for the fact that egg hatching is not strictly synchronized among individuals in a randomly collected cohort of animals isolated at birth. Instead, individuals generally hatch their eggs sometime over a defined period of egg development. Since this period is usually longer than the period between observations, different fractions of ovigerous females are expected to hatch their eggs during different age classes.

We assumed that F_x is distributed normally with mean μ^* and variance σ^{2*}. Instead of specifying a common variance for all age classes, we assumed that the coefficient of variation of $F_x(k)$ is constant across all age classes. Mean clutch size μ^* is age-specific, as is explained later in Eq. 14. Therefore, the age-specific variance becomes $(k\mu^*)^2$. This pattern corresponds to our observations of the freshwater cladoceran *Daphnia pulex*. Using the algorithm of Abramowitz and Stegun (1964), we truncated the random variable F_x at zero and twice the mean yielding a symmetrically truncated normal distribution that we denote as

$$(9) \qquad F_x \sim N_T[\mu^*, (k\mu^*)^2].$$

The degree of truncation (i.e. the number of random variables rejected) is less than 1% when $k < 0.3$ but increases to about 20% when $k = 0.6$. Our observations of *D. pulex* clutch sizes indicated that k was usually less than 0.3; thus the truncated normal distribution is a useful approximation.

We predicted mean clutch size as a function of age (μ_x) using three alternative reproductive strategies (Fig. 2). These strategies apply to mature females in the age-class interval $[x_r, A]$ where x_r is the age class at first birth and A is the maximum number of age classes. In the first strategy, type A, mean clutch size increases as a power function over the life of the animal:

$$(10) \qquad \mu_{A,x} = \alpha(x\Delta a)^{\beta}$$

where α and β are constants. For type B reproduction, clutch size increases with age but approaches an asymptotic value:

$$(11) \qquad \mu_{B,x} = \mu_{\max}[1 - \exp(\gamma x\Delta a)]$$

where γ and $\mu_{\max}$ are constants. For the third strategy, type C, reproductive effort is concentrated in the period immediately following the onset of sexual maturity. Older animals, however, have a long period of decreasing reproductive output followed by senescence:

$$(12) \qquad \mu_{C,x} = \theta(x\Delta a)\exp(\psi x\Delta a)$$

where θ and ψ are constants. To make valid comparisons of alternative reproductive strategies, we selected the parameter constants of Eq. 10 to 12 so that mean clutch sizes integrated over the reproductive age-class interval $[x_r, A]$ equal a constant value C. Denoting age with a instead of $x\Delta a$, we therefore required that

$$(13) \qquad \int_{x_r\Delta a}^{A\Delta a} \mu_{A,x}(a)da = \int_{x_r\Delta a}^{A\Delta a} \mu_{B,x}(a)da = \int_{x_r\Delta a}^{A\Delta a} \mu_{C,x}(a)da = C.$$

Another source of variation in reproduction is related to the distribution of reproductive effort over the egg-development period (Δe). Unless a cohort is completely synchronized, individual females will generally produce their clutch sometime during the time interval $x\Delta a$ to $x\Delta a + \Delta e$, where $\Delta e = h\Delta a$ and h is an integer for our simulations. By denoting the fraction of females in age class x that hatch offspring as f_x, where $0 \leq f_x \leq 1$, and by assuming that all females that survive the interval $x\Delta a$ to $x\Delta a + \Delta e$ reproduce, then

$$\sum_{x=1}^{h} f_x = 1.$$

This set of assumptions implies that f_x is a random variable from a multinomial distribution with parameters $(h, q_x, q_{x+1}, \ldots, q_{x+h-1})$ where $E(q_x) = h^{-1}$ for all x.

We computed f_x by generating a sequence of uniformly distributed random numbers with range [0, 1] for each age class from the age class at first birth (x_r) to the maximum age class (A). For each h-tuple of age classes beginning at x_r, the random numbers are summed and each element of the h-tuple of age classes is divided by this sum. The resulting sequence of numbers consists of consecutive sets of h fractions each summing to one.

In Eq. 10 to 12 we describe how mean clutch size varies with age. *Daphnia pulex* and other Cladocera, however, allocate their net assimilate to reproduction over the entire period of egg development (Dorazio and Lehman 1983; Tessier et al. 1983). The average size of clutches hatched by animals over any single egg-development period Δe should therefore be identical for all animals in each h-tuple of age classes. We computed this average clutch size by randomly weighting mean clutch sizes predicted by Eq. 10 to 12 by the fractions of reproductive females from each set of h age classes:

$$\mu^* = \sum_{j=1}^{h} f_{x+j-1}\mu_{x+j-1}. \quad (14)$$

A separate, common mean clutch size μ^* is therefore computed for each period of egg development Δe. In the model the realized mean clutch size of animals in age class x, F_x, is randomly drawn from a truncated normal distribution with mean μ^* and variance $(k\mu^*)^2$, as described earlier (Eq. 9). The mean number of offspring produced by animals in age class x is then computed from

$$m_x = f_x F_x. \quad (15)$$

Thus, m_x is the product of two random variables, one drawn from a truncated normal distribution and the other drawn from a multinomial distribution.

Simulation Experiments of Hypothetical Cohorts

To examine the influence of survival pattern, reproductive type, and cohort size on the sampling distribution of λ, we simulated a three-way experiment composed of three different survival patterns, three reproductive types, and five cohort sizes. For survival the maximum age was fixed at 48 d, and c was set to 1.0, 2.5, and 5.0. Reproductive types A, B, and C were described earlier. Animals hatched their first clutch on day 10 in all simulations. We considered a range of cohort sizes that might be used in actual experiments (5, 10, 20, and 40 animals) and we computed 1000 values of λ for each combination of parameters. Comparing results of preliminary simulations in which the number of iterations was varied from 100 to 10 000 indicated that 1000 iterations sufficiently characterized the sampling distribution of λ. Initialization of each simulation required specification of the number of iterations, cohort size, maximum age class, duration of each age class, shape parameter for survivorship, and the functional form of the reproductive pattern and resetting the seed for generation of random numbers. The same random-number seed was used for each simulation to insure comparability between runs and reproducibility of a given run. The finite rate of increase λ was computed from the random vectors l_x and m_x by combining the Half-interval method with Newton's method (Carnahan et al. 1969).

We examined the asymptotic properties of the Monte Carlo model by computing values of λ using a cohort size of 1000 individuals. Our objective was to compare these results with the sampling distribution characteristics of λ based on much smaller cohort sizes. Due to the excessive computer time required for these simulations, we only considered cases that led to large variability in distributions of λ with small cohort sizes.

Application to *Daphnia pulex*

The Monte Carlo model can be applied to real data in a variety of ways. Specific models of survival and reproduction could be specified, parameterized, and used as described earlier. Alternatively, an empirical approach can be taken in which the actual variability of the data set is used directly to parameterize the underlying distributions of N_x and F_x. We used the latter approach and drew random values of N_x and F_x from their estimated underlying distributions. Life-table experiments designed to examine the effects of pH on cohorts of *D. pulex* were conducted over a period of 100 d (P. J. Rago, unpubl. data). Twenty *D. pulex* were cultured individually in 25-mL vials at pH 7.5, 6.5, 6.0, and 5.5 until all individuals died. Animals were raised in a buffered artificial medium (Lehman 1976). Each day, animals were transferred to new vials containing 10^5 cells·mL^{-1} of the alga *Chlamydomonas reinhardi*. Survival and numbers of offspring produced by each individual animal were recorded daily. We estimated the l_x curve by smoothing the trajectory of survivorship over time using a centered 5-point moving average. We employed this approach because the Weibull model did not fit the data very well. Specifically, mortality seemed to be concentrated in the period before sexual maturity and in a senescent period near the maximum age. This pattern is often observed in tests of material lifetimes (Hahn and Meeker 1982), but to our knowledge a single continuous model for such patterns has not been proposed. The moving-average approach allowed us to obtain a smoothed representation of the data without specifying an underlying model. To generate a random l_x curve, we computed age-specific survivorships p_x from Eq. 4 and combined these with cohort size N_0 to parameterize the chain binomial model (Eq. 6, 7) for N_x. The realized l_x curve was then computed from the distribution of N_x by Eq. 8. We characterized reproduction in each age class by computing the sample mean $\hat{\mu}_x$ and variance $\hat{\sigma}_{F_x}^2$ of clutch sizes measured for animals that produce offspring. Values of F_x, mean clutch size of animals in age class x, were randomly drawn from truncated normal distributions parameterized by our estimates $\hat{\mu}_x$ and $\hat{\sigma}_{F_x}^2$. Random values of age-specific fecundity, m_x, were then computed as the product of F_x and the fraction of animals in age class x that produced offspring, f_x. For each treatment pH we computed 1000 random values of λ to approximate its sampling distribution.

Results and Discussion

Distributional Trends of λ for Hypothetical Cohorts

The magnitude of a population's finite rate of increase depends on the schedules of reproduction and mortality that are characteristic of its members. The accuracy of any single estimate of λ therefore depends on one's ability to estimate the set of life-history traits that is representative of an average group of animals from the population. The number of animals that make up a life-table experimental cohort therefore plays an important role in determining the experimental value of λ. Our simulations, in fact, showed that cohort size strongly affects the qualitative shape of sampling distributions of λ generated for hypothetical populations of animals that experience a wide variety of l_x and m_x schedules. In Fig. 3 we illustrate the effects

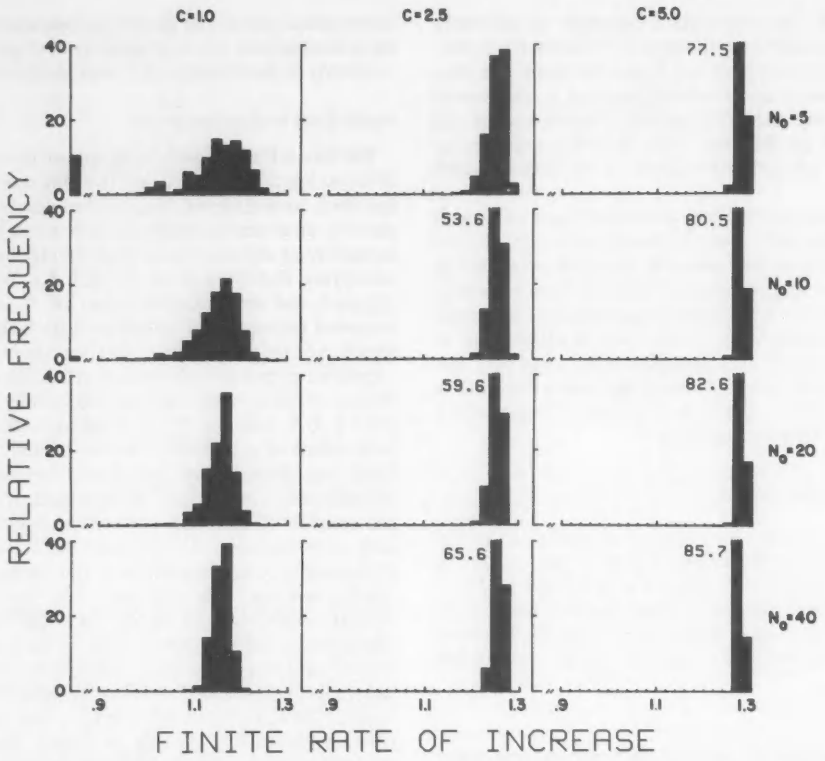

FIG. 3. Sampling distributions of λ generated for hypothetical populations; c refers to the Weibull shape parameter and N_0 refers to cohort size. All animals display type B reproduction with $k = 0.15$.

of cohort size on λ by plotting four distributions for each l_x schedule corresponding to four cohort sizes. These cohort sizes span the range commonly used in life-table experiments with zooplankton. Reglardless of the pattern of age-specific mortality experienced by the animals, a single distributional trend is common. At small cohort sizes the distribution of λ is skewed toward lower values and can, in some cases, be bimodal. As cohort size increases, bimodality is eliminated, the distribution becomes more symmetrical, and the variance of λ decreases. These trends also exist for animals with types A and C reproductive strategies. Sampling distributions of λ for these cases are not shown here.

Bimodal distributions of λ arise only when mortality is concentrated on juveniles (e.g. when the Weibull shape parameter $c = 1.0$) and cohort size is small. Under these conditions there is a relatively high probability that all animals from the cohort die before they produce offspring; λ becomes zero in this case. As the initial number of animals in the experimental cohort increases, however, the probability that at least one of these animals survives to become reproductively mature increases, and zero values of λ are eventually eliminated. Skewed distributions of λ are most conspicuous when animals are exposed to high mortality early in life ($c = 1.0$) or when cohort size is small. Reductions in age-specific survivorship *early* in life tend to decrease the value of λ to a greater extent than if similar reductions were experienced *later* in life because individual contributions to λ are negatively exponentially weighted with respect to age (cf. Eq. 1; Birch 1948; Cole 1954). Therefore, when a large proportion of the population dies early in life, either because mortality is highest on young individuals or because there were few animals to start with, λ is reduced to a greater extent than if these animals had died when they were older. This differential weighting by age is manifested in distributions of λ by a higher frequency of low values, which results in skewing and asymmetry. As cohort size increases, however, the probability that a large proportion of the population will die early in life decreases, and distributions of λ become more symmetrical. Even with large cohorts, though, the sampling distribution remains skewed; in all but 1 of the 36 simulated distributions for the hypothetical populations, the mean was less than the median value of λ. For each reproductive strategy the difference between the median and mean decreased as cohort size increased and as c increased from 1.0 to 2.5 to 5.0.

These results have obvious implications for hypothesis testing. Since skewed sampling distributions of λ are often generated with commonly used cohort sizes, normal-based statistical procedures may be misleading. We recommend examining the entire sampling distribution of λ when making comparisons of values of λ determined independently in separate experiments. In a later section of this paper, we demonstrate how this examination can be accomplished using experimentally measured l_x and m_x schedules.

Altering schedules of reproduction produces less dramatic changes in the distribution of λ than those described above. The means and modes of distributions generated for populations of

TABLE 2. Means from sampling distributions of λ generated for hypothetical cohorts with three reproductive strategies (A, B, C), three mortality schedules (c = 1.0, 2.5, 5.0), and six cohort sizes (N_0 = 5, 10, 20, 40, 80, 1000). Deterministic values of λ computed from expected l_x and m_x schedules are also listed. Coefficient of variation in clutch size (k) equals 0.15 in all cases. NC = not computed.

	Type A			Type B			Type C		
N_0	1.0	2.5	5.0	1.0	2.5	5.0	1.0	2.5	5.0
5	0.8622	1.0862	1.1256	1.0361	1.2531	1.2748	1.1630	1.4107	1.4304
10	0.9869	1.0904	1.1259	1.1419	1.2535	1.2747	1.2805	1.4104	1.4303
20	1.0214	1.0941	1.1263	1.1564	1.2536	1.2748	1.2963	1.4108	1.4317
40	1.0366	1.0952	1.1261	1.1591	1.2543	1.2738	1.2983	1.4134	1.4293
80	1.0419	NC	NC	1.1594	NC	NC	1.2992	NC	NC
1000	NC	NC	NC	1.1606	1.2554	1.2742	NC	NC	NC
Deterministic	1.0459	1.0945	1.1253	1.1600	1.2540	1.2741	1.2934	1.4039	1.4227

animals that produce large clutches early in life are greater than those for populations of animals that produce their largest clutches later in life (see Table 2). Distributions of λ generated for populations of animals whose clutch sizes approach an asymptotic value with age have means and modes intermediate to these two cases. The relative importance of the youngest fecund females in determining the magnitude of λ (see discussion above) is responsible for these distributional trends. The hatching of large clutches early in life leads to increases in the values of λ. For females with similar mortality schedules, a change in schedule of reproduction therefore affects the mean and mode of the distribution of λ but not the pattern of skewness, which is primarily determined by cohort size and age-specific survivorship.

Compared with the effects of changing age-specific schedules of mortality and fecundity, altering the relative variation in clutch size produces only minor effects on distributions of λ. Increases in the coefficient of variation in clutch size from 0.15 to 0.3 to 0.6 result in an increase in the variance of λ (Fig. 4), but the increase is small relative to that produced by lowering cohort size or survivorship.

Asymptotic Approximations of Distributions of λ

Previously, we demonstrated that increasing the initial number of animals in an experimental cohort tends to make the distribution of λ more symmetrical and reduce its variance. Here we examine whether the distribution of λ asymptotically approaches a normal distribution as cohort size becomes very large. We examined three examples that correspond to the three mortality schedules used previously. In each example the cohort was composed of 1000 animals, and to eliminate effects of fecundity schedule on λ, a single clutch size function (type B) and coefficient of variation (0.15) are used.

When we applied the Kolmogorov–Smirnov goodness-of-fit test to these asymptotic distributions of λ they were significantly non-normal ($P < 0.01$ for l_x schedules corresponding to Weibull shape parameter (c) values of 1.0 and 2.5, and $0.05 < P < 0.10$ for an l_x schedule corresponding to $c = 5.0$). These results are important because they emphasize that normal-based procedures are inappropriate for hypothesis testing of values of λ even when cohort size is very large. As for the case of small cohort sizes, the entire sampling distribution of λ must be considered when one wants to compare values of λ obtained independently in separate experiments.

These large-cohort simulations do show, however, that the mean of sampling distributions of λ converges to the value of λ that corresponds to the underlying processes responsible for the observed age schedules of reproduction and mortality. Table 2 contains the means of sampling distributions of λ generated from Monte Carlo simulations and values of λ computed strictly from l_x and m_x schedules that correspond to the Weibull distribution functions and clutch size functions that we used. As cohort size increases, the means of sampling distributions of λ converge to deterministic predictions of λ. We therefore recommend using large cohorts in life-table experiments to reduce variability in the sampling distribution *and* to decrease bias of the mean λ. Our finding of bias as a function of cohort size agrees with results of Lenski and Service (1982).

Empirical Sampling Distribution of λ

We have thus far limited our discussion to sampling distributions of λ generated for populations of animals experiencing hypothetical mortality and fecundity schedules. Here we show how sampling distributions of λ generated from experimentally measured l_x and m_x schedules can be used in hypothesis testing. Four life-table experiments were conducted to determine the effects of pH on the life history of *D. pulex*. Twenty animals were used at each treatment pH. Sampling distributions of λ for each treatment are shown in Fig. 5. Variations in the shapes of the sampling distributions are due solely to the effects of changes in the l_x and m_x curves. At pH 7.5, survival followed a Type I pattern and brood sizes were consistently high for the first several reproductive periods. At pH 6.5 and 6.0, neonates experienced high mortality prior to sexual maturity but limited additional mortality until they approached their maximum age. Relative to the 6.5 distribution, the sampling distribution for the pH 6.0 cohorts is shifted to lower values of λ because brood sizes were consistently smaller. At pH 5.5, prereproductive mortality was high, brood sizes were small, and the maximum age was greatly decreased. For those comparisons in which the ranges of these distributions do not overlap, there is little doubt that values of λ are significantly different. The finite rate of increase of populations of *D. pulex* raised at pH 5.5, for example, is always lower than the minimum value of λ that could be achieved at any of the higher pH treatments. In other comparisons the ranges of these distributions overlap, and a probability statement must be used to distinguish significant differences in λ for any pairwise comparison.

As described earlier, sampling distributions of λ are not, in general, normal. If a closed-form expression for the variance of

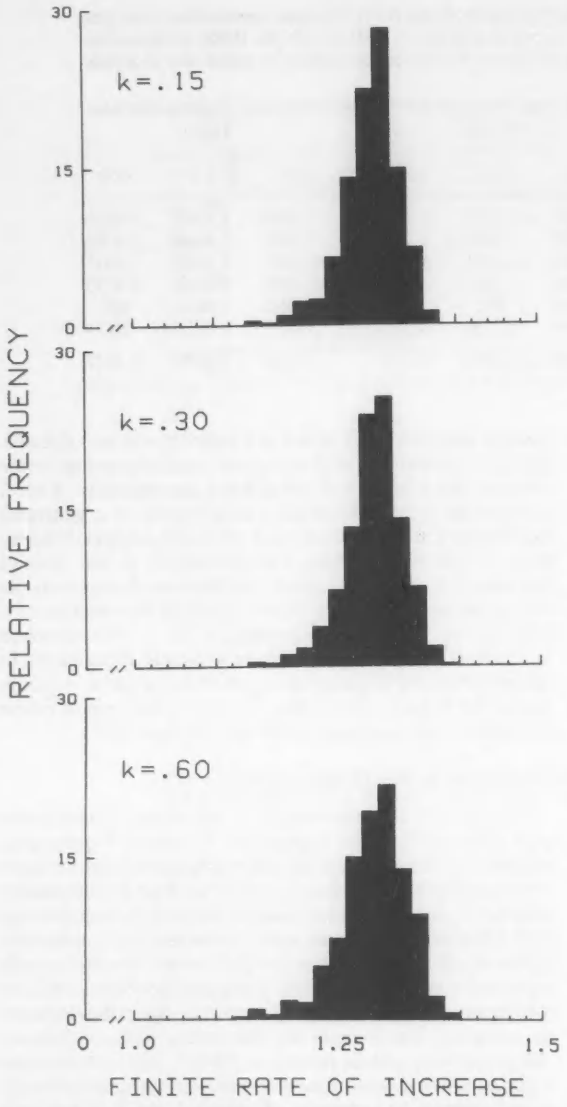

FIG. 4. Sampling distributions of λ generated for hypothetical populations; *k* refers to the fractional coefficient of variation in clutch size. Each hypothetical cohort consists of 20 animals that display type C reproduction and experience a survivorship curve characterized by $c = 1.0$.

λ were available, it would only be an approximation and its use could be misleading in terms of statistical inference (e.g. Lenski and Service 1982; but see below).

We recommend comparing pairs of sampling distributions of λ directly by specifying one as the null distribution and the other as the alternative. Having done so, we can proceed by setting a level of significance α, determining critical values of λ, and estimating the resulting probability of Type II errors (Table 3). Having approximations of both the null and alternative distributions, however, permits inferences not afforded by traditional methods. With both distributions it is possible to obtain confidence regions that minimize the probability of a Type II error for a specified level of Type I error. In other words, confidence intervals can be set asymmetrically to satisfy desired levels of risk. Furthermore, with both distributions it is possible to examine the region of overlap between the distributions and assess the relative uncertainty for values of λ in this region. Thus the relative probability that a specific value of λ belongs to the null distribution and not to a specific alternative can be assessed. We defined this relative probability as ω; its derivation follows. Consider two distributions of λ, *A*, and *B*, whose ranges overlap. Let $p_A(\lambda)$ be the continuous probability density function for distribution *A* and $p_B(\lambda)$ be the pdf of distribution *B*. Let $\tilde{p}_A(\lambda_0)$ define the probability that λ is in the neighborhood of λ_0, say $\lambda_0 \pm \epsilon$ where ε is some arbitrarily small value. In integral form

$$\tilde{p}_A(\lambda_0) = \int_{\lambda_0-\epsilon}^{\lambda_0+\epsilon} p_A(\lambda)d\lambda.$$

In the region where distributions *A* and *B* overlap, the probability that λ_0 belongs to distribution *A* and not *B* can be defined as

$$\omega = \frac{\tilde{p}_A(\lambda_0)}{\tilde{p}_A(\lambda_0) + \tilde{p}_B(\lambda_0)}. \quad (16)$$

In this way, $\omega = 1.0$ when λ_0 belongs to *A* but not *B* and $\omega = 0.0$ when the opposite is true. Thus one can examine ω as a function of λ in the region of overlap. Furthermore, the confidence region can be defined as that interval of λ in which 100ω% or more of the values of λ are from the null distribution and not the alternative. When actually computing ω it is important that ε be chosen such that small irregularities in the region of overlap between sampling distributions are reduced. In our simulations a sample size of 1000 random values of λ proved satisfactory. Values of ω computed in a comparison of life-table experiments at pH 6.5 and 6.0 are illustrated in Fig. 6. The sampling distributions of λ for these two treatments overlap considerably (Fig. 5); however, we used numerical searching procedures to determine that $\omega > 0.95$ for $\lambda > 1.315$ and λ less than the maximum of the sampling distribution corresponding to pH 6.5 (i.e. $\lambda = 1.375$). Therefore, we can say that we are at least 95% certain that values of λ in the interval {1.315, 1.375} may be attributed to raising *D. pulex* at pH 6.5 and not pH 6.0. Notice that this interval comprises only about one half of the entire sampling distribution of λ associated with pH 6.5. The large degree of overlap between this distribution and that corresponding to pH 6.0 therefore limits the range of λ over which we can detect differences in λ due to pH. Also, see Table 3.

Our procedure for making pairwise comparisons of sampling distributions is a general method. No assumptions are made regarding the shape of the distributions or their moments. Although many simulated values of λ are required to adequately resolve each sampling distribution, this limitation is easily overcome with current computing facilities.

Comparisons with Other Variance Estimators of λ

A central theorem in inferential statistics is the relationship between the population variance and the variance of the sampling distribution of the mean. In particular, if samples of size *N* are drawn from an underlying distribution, then the variance of the means of these samples ($\sigma_{\bar{x}_N}^2$) will be equal to the population variance divided by the sample size (σ^2/N). The usual line of inference is to draw a sample of size *N*, compute the sample mean, and find an unbiased estimate of σ^2, say s^2. Since

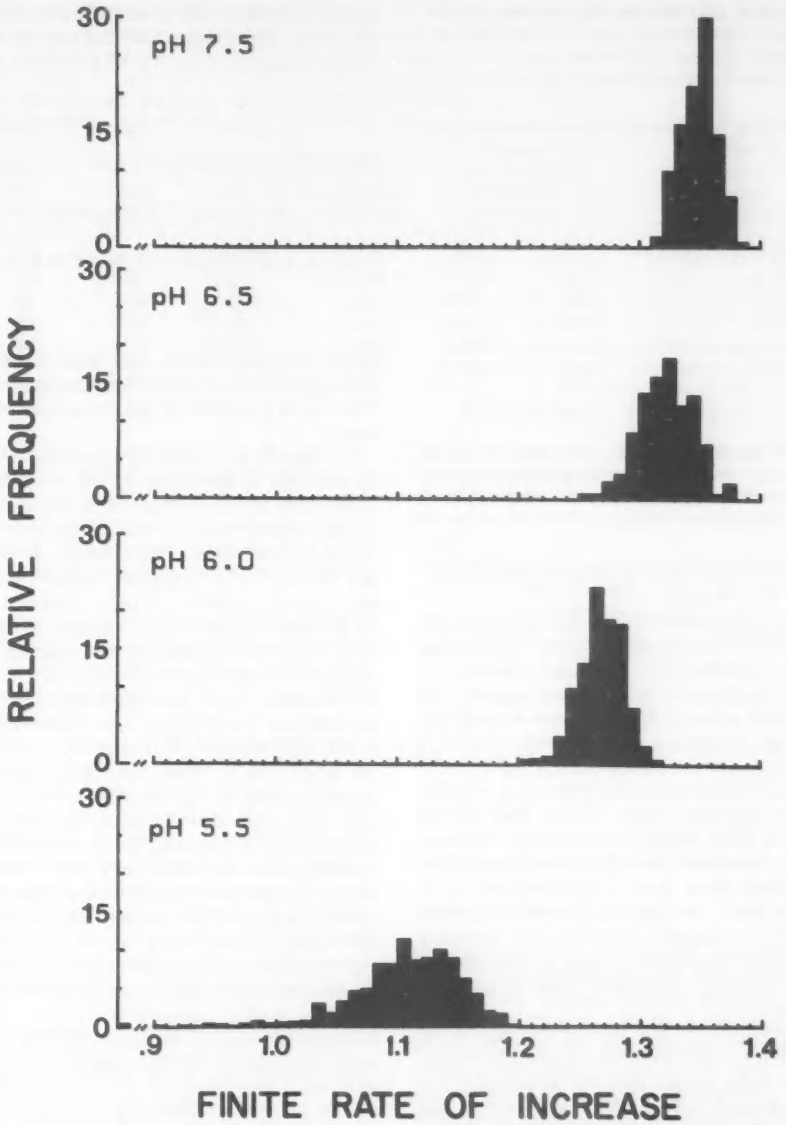

FIG. 5. Sampling distributions of λ generated for experimental cohorts of *D. pulex* raised at four different pH's. Cohorts consist of 20 animals in each case.

TABLE 3. Probabilities of Type II error for two-sided hypothesis test ($\alpha/2 = 0.025$) for *D. pulex* experiments. Letter in parentheses indicates whether upper (U) or lower (L) bound is source of overlap.

		pH			
pH	95% confidence interval	7.5	6.5	6.0	5.5
7.5	[1.3221, 1.3746]	—	0.496(L)	0	0
6.5	[1.2755, 1.3655]	0.886(U)	—	0.345(L)	0
6.0	[1.2302, 1.3008]	0	0.158(U)	—	0
5.5	[0.9736, 1.1742]	0	0	0	—

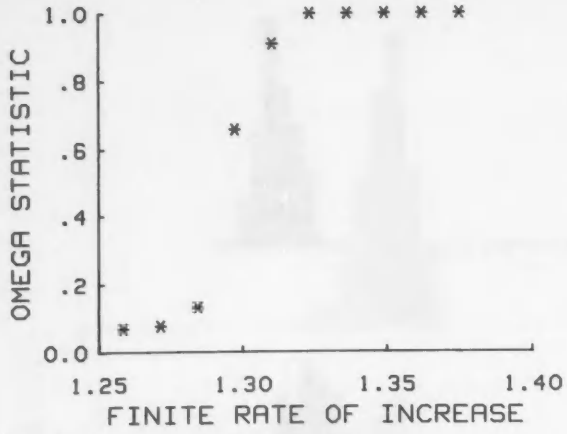

FIG. 6. Comparison of sampling distributions of λ generated for *D. pulex* cohorts raised at pH 6.5 and 6.0 using the ω statistic (Eq. 16). The empirical sampling distribution for animals raised at pH 6.5 corresponds to the null distribution.

$E(\bar{x}) = \mu$ and $E(s^2) = \sigma^2$, inferences about μ are made using $\bar{x}$ and s^2 assuming that $s^2/N = \sigma_{\bar{x}_N}{}^2$.

Lenski and Service (1982) demonstrated that a finite rate of increase could be computed for the ith individual in the cohort (λ_i) if a common λ_N, estimated for the entire cohort of N individuals, was used to discount the individual reproductive schedules. The expected value of λ_i was shown to equal the population growth rate. Moreover, the expected value of a variance estimator of λ, say $s_L{}^2$, was shown to be biased but this bias could be reduced by either increasing cohort size or using a jackknife-estimation approach. Later, Lenski and Service (1982) made inferences about sample populations by assuming that the mean values of λ_N were normally distributed and that $s_L{}^2/N = \sigma_{\lambda_N}{}^2$. Our results show that the distribution of λ_N is significantly different from a normal distribution for a wide variety of commonly encountered mortality and fecundity schedules. Hence, statistical inferences based on assumptions of normality could be imprecise, especially when distributions of λ_N are highly skewed.

A more disturbing comparison was that the variance of the mean ($\sigma_{\lambda_N}{}^2$) computed from our simulated distributions for the *D. pulex* cohorts was much smaller than that predicted by the Lenski and Service estimator (i.e. $s_L{}^2/N$). To investigate this inconsistency, we examined the sampling distribution for the ideal population of Lenski and Service (1982, table 1). They defined their estimator in terms of "lives," which were defined as a unique collection of reproductive events (i.e. successive broods) terminating with the death of the individual. A population comprises a set of individuals following distinct lives. In an ideal population the number of different lives is finite, say K, and several individuals can follow life k, say n_k, with probability π_k. In a sample of size N drawn from this ideal population, the expected number of individuals following life k is equal to $N\pi_k$ and the distribution of individuals in various lives will follow a multinomial distribution with parameters (N, $\pi_1, \pi_2, \ldots, \pi_K$) (Johnson and Kotz 1969). If the equations of Lenski and Service are modified slightly, one can write Lotka's (1907a, 1907b) equation in terms of individual lives as follows.

Let $B_{x,k}$ equal the number of offspring produced by an individual in age class x following life k. By definition, $B_{x,k}$ equals zero when individuals following life k die. Let $\bar{S}_x$ equal the proportion of individuals that survive to age class x. Using these definitions, Lotka's (1907a, 1907b) equation becomes

$$(17) \qquad 1 = \sum_{x=1}^{A} \hat{\lambda}_N{}^{-x} \bar{S}_x \left(\sum_{k=1}^{K} n_k B_{x,k} \right) \Big/ (N\bar{S}_x)$$

which upon simplification yields

$$(18) \qquad 1 = \sum_{x=1}^{A} \hat{\lambda}_N{}^{-x} \sum_{k=1}^{K} (n_k/N) B_{x,k}$$

where $\hat{\lambda}_N$ is an estimate of λ_N based on a cohort of N animals and

$$N = \sum_{k=1}^{K} n_k.$$

Using this formulation, the random variables are not the demographic events of survival and reproduction but are the n_k. Thus, $\hat{\lambda}_N$ is a function of the frequencies of various lives in the sample.

To examine the validity of Lenski and Service's estimator of the variance of the mean, $s_L{}^2/N$, we generated the sampling distribution of $\hat{\lambda}_N$ for their ideal population (cf. Lenski and Service 1982, table 1). Simultaneously, we simulated the sampling distribution of the variance estimator $s_L{}^2$ using Lenski and Service's (1982) formula (their Var (F_i')) and a Taylor series approximation ($s_T{}^2$) to be described in the next section. As we observed before, the variance of the sampling distribution of $\hat{\lambda}_N$ was significantly less than the mean value of the Lenski–Service estimator divided by N (i.e. $s_L{}^2/N$) (see Table 4). The mean $\hat{\lambda}_N$ and the mean of the variance estimates ($s_L{}^2$) of the simulated distributions were, however, equal to those that Lenski and Service (1982) calculated for their ideal population (cf. their table 1). Thus, the discrepancy was not due to any systematic bias in our simulations of $\hat{\lambda}_N$ and $s_L{}^2$ but to the nonlinearity of Eq. 18 itself. Kendall and Stuart (1977) demonstrated that the variance of nonlinear functions of random variables does not necessarily bear a direct relation to the variance of the mean. Since $\hat{\lambda}_N$ is a nonlinear implicit function of the n_k in Eq. 18, it is unlikely that an explicit formulation for the variance of the mean can be found. As an alternative, a Taylor series expansion can be used to estimate the variance of the mean directly (Kendall and Stuart 1977).

Let the random vector $\bar{n} = (n_1, n_2, \ldots, n_K)$ and $g(\hat{\lambda}_N, \bar{n})$ represent the version of Lotka's (1907a, 1907b) equation that we presented in Eq. 18. The variance of $\hat{\lambda}_N$ can be approximated as

$$(19) \qquad \text{Var}(\lambda_N) \simeq s_T{}^2 = \sum_{k=1}^{K} D_k{}^2 \,\text{var}(n_k) + \sum_{i \neq j}^{K} \sum_{j \neq 1}^{K} D_i D_j \,\text{cov}(n_i, n_j)$$

where var $(n_k) = n_k(N - n_k)/N$ and cov $(n_i, n_j) = -n_i n_j/N$ (Johnson and Kotz 1970). The D_k are the partial derivatives of λ_N with respect to n_k evaluated at $\hat{\lambda}_N$ and $\hat{n}_k$, $k = 1, 2, \ldots, K$. We denote the realized number of animals experiencing life k by $\hat{n}_k$ (See Appendix A for a derivation.).

To test this approximation, the sampling distributions of $\hat{\lambda}_N$, $s_L{}^2$, and $s_T{}^2$ for the ideal population of Lenski and Service were examined for cohort sizes of 5, 10, 20, 40, 80, and 1000 animals (Table 4). For each cohort size, the average $\hat{\lambda}_N$ was very close to the underlying true population value of 1.1483 and the average of $s_L{}^2$ was very close to the true population value of 0.4813. However, the predicted average variance of the mean, defined

TABLE 4. Comparison of the Lenski and Service (LS) variance estimator, a Taylor series (TS) variance estimator, and actual variances computed for simulated sampling distributions of $\hat{\lambda}_N$. Results are based on 1000 simulated values drawn from the ideal population of Lenski and Service (1982, table 1), which had a mean $\lambda = 1.1483$ and a variance = 0.4813. Percent deviations of the LS and TS variance estimates from the simulated variances of the mean are in parentheses.

Cohort size (N)	Average of $\hat{\lambda}_N$	Simulated variance of the mean	Average LS estimate of variance	Predicted LS estimate of variance of the mean		Average TS estimate of variance of the mean	
5	1.1435	0.0607	0.4939	0.0988	(+63%)	0.0458	(−25%)
10	1.1415	0.0302	0.4962	0.0496	(+64%)	0.0261	(−14%)
20	1.1517	0.0142	0.4825	0.0241	(+70%)	0.0135	(−5%)
40	1.1492	0.00726	0.4861	0.0122	(+68%)	0.00695	(−4%)
80	1.1493	0.00351	0.4849	0.00606	(+73%)	0.00360	(+3%)
1000	1.1478	0.000260	0.4842	0.000484	(+86%)	0.000284	(+9%)

as s_L^2/N, was considerably greater (about 65%) than the variance of the $\hat{\lambda}_N$ computed from the simulated distribution of $\hat{\lambda}_N$. In contrast, the averages of the s_T^2 were much closer to the computed variances for all cohort sizes. Thus, it appears that the multinomial model and Taylor series expansion are valid approximations for populations consisting of only K different lives.

In a real population there are, of course, an infinite number of lives rather than K lives as noted by Lenski and Service (1982). The multinomial model and Taylor series expansion are still appropriate. Without loss of generality, it can be assumed that each life (i.e. individual) in a cohort is distinct (i.e. $n_i = 1$, $i = 1, 2, \ldots, N$). This amounts to having N variance terms rather than K and $N^2 - N$ covariance terms in Eq. 19 (see Appendix B). As cohort size increases, the variance terms approach 1 and the covariance terms approach 0. In Appendix B we show that s_T^2 for N lives is equivalent to s_T^2 for K lives if, in fact, there are only K distinct elements in the partial derivatives. In practice, data on reproductive output for each individual can be used directly in the Taylor series expansion by using Eq. A2.2. When we did this for the *D. pulex* data, s_T^2 was between 60 and 200% of the variance of the simulated means, whereas s_L^2 overestimated the simulated variance by as much as two orders of magnitude (Table 5).

In terms of statistical inference, these results mean that the precision of estimates of λ_N is much better than indicated by the results of Lenski and Service (1982). Their approach is far more conservative because only very large differences in λ_N can be judged as significantly different. The penalty for this conservative approach is that the probability of Type II statistical errors (stating that no significant differences exist when in fact they do) becomes very large. Hence, the subtle effects one wishes to discern in a life-table test are less detectable. Since we have shown that the distribution of $\hat{\lambda}_N$ is non-normal for a wide variety of l_x and m_x schedules and since the simulation approach provides considerably more insight into the factors causing the underlying variation and permits direct estimation of Type I and II errors, the Taylor series estimator should be considered only when the Monte Carlo estimation procedures are unavailable. Since the Taylor series approximation appears to underestimate the true variance, the resulting confidence intervals are smaller than predicted by the normal-based critical value (Table 6). For example, $t_{19,0.975} = 2.093$ yields a confidence interval for the pH 7.5 treatment that includes only 87% of the simulated values. To obtain an estimated 95% confidence interval for this case, a critical value of t of about 2.411 with a probability level of about 0.985 is required. In contrast, the Lenski–Service estimator yields a confidence interval that exceeds even the range of the distribution. These results illustrate the shortcomings of combining approximate variance estimators, such as s_T^2 and s_L^2, with normality assumptions. In view of the skewness of λ_N, Chebyshev confidence intervals (Steel and Torrie 1960) using s_T^2 might be more useful when the sampling distribution is not simulated.

TABLE 5. Comparison of simulation results for *D. pulex* cohorts with the Taylor series approximation and the Lenski and Service (1982) estimator. Numbers in parentheses equal percent deviation of the variance estimate from the simulated variance of the mean based on 1000 cases.

	Simulation results			
pH	Average λ_N	Variance of λ_N	Taylor series s_T^2	Lenski-Service s_L^2/N
7.5	1.3484	2.0145×10^{-4}	1.1846×10^{-4} (−41.2%)	3.1392×10^{-2} (15 483.0%)
6.5	1.3240	4.9300×10^{-4}	3.5370×10^{-4} (−28.3%)	6.3262×10^{-2} (1 273.2%)
6.0	1.2690	3.3380×10^{-4}	2.7386×10^{-4} (−18.0%)	6.0979×10^{-2} (1 790.4%)
5.5	1.1231	7.2467×10^{-4}	1.4340×10^{-3} (97.8%)	2.4550×10^{-1} (33 777.5%)

Relevance to Experimental Design

The simulation results have important implications for the design and analysis of life-table experiments. We have shown that the distribution of λ is often skewed, is significantly different from a normal distribution even when cohort size is large, and can even be bimodal. By providing insight into the specific mechanisms that give rise to these distributional characteristics, simulations not only aid in interpretation of existing data but can assist in the design of future experiments.

Generation of the sampling distribution of λ under various experimental treatments permits a very powerful analysis of results, as we have shown. This approach, however, requires a considerable amount of numerical calculation, and since the Taylor series variance approximation yields a relatively accu-

TABLE 6. Comparison of predicted 95% confidence intervals with 95% confidence interval for simulated values of $\hat{\lambda}_N$. Number in parentheses is the fraction of simulated values within the predicted confidence interval. Predicted confidence intervals assume that $\hat{\lambda}_N$ is normally distributed $t_{19,0.975} = 2.093$.

		Predicted interval	
pH	Simulation results	Taylor series estimate	Lenski and Service estimate
7.5	[1.3221, 1.3746]	[1.3256, 1.3711] (0.869)	[0.9775, 1.7192] (1.00)
6.5	[1.2755, 1.3665]	[1.2846, 1.3634] (0.926)	[0.7976, 1.8504] (1.00)
6.0	[1.2302, 1.3008]	[1.2344, 1.3036] (0.937)	[0.7522, 1.7858] (1.00)
5.5	[0.9736, 1.1742]	[1.0439, 1.2024] (0.905)	(0.0861, 2.1602] (1.00)

rate estimate of the variance of the mean, it is reasonable to consider application of more traditional methods. Analysis of variance (ANOVA) is one possibility (e.g. Winner et al. 1977; Service and Lenski 1982). Although ANOVA is relatively insensitive to non-normality, it is sensitive to the assumption of homogeneity of variance (Green 1979). Our results show that changes in mortality and fecundity schedules not only alter the mean λ but also its variance. Moreover, it is often difficult to maintain a balanced design because animals may die due to handling stress or other factors not related to controlled experimental conditions. When this occurs in two-way and higher order layouts, ANOVA comparisons become nonorthogonal and difficult to handle. Thus, we argue that ANOVA is inappropriate for analysis of λ. Nonparametric methods might be useful, but to our knowledge no research on their use has been done.

Selecting the appropriate cohort size in life-table experiments is always a concern. One criterion that has been advanced by the American Society for Testing and Materials (Proposed Standard Practices — Draft 7, cited by Goulden et al. 1982a) is that 80% of the control population survives the first 21 or 28 d of a life-table experiment. Goulden et al. (1982a) used a binomial model to select a cohort size that would reduce the probability of violating this criterion to 0.01 for the control cohort. The simulation approach permits one to expand this criterion further by explicitly considering probabilities of Type I and II errors for each experimental treatment. If expected results of the treatment on survivorship and fecundity can be hypothesized or are known from previous studies, it should be possible to predict expected differences in the distributions of $\hat{\lambda}_N$ and select appropriate cohort sizes that reduce both types of statistical errors. As an example, suppose a treatment reduces brood size at each age by 40% but has no effect on survival. Distributions of $\hat{\lambda}_N$ could be generated for various sample sizes, and the probabilities of detecting true differences assessed. Since a balanced experimental design is not required for the empirical comparisons of sampling distributions, equal cohort sizes for each treatment are not necessary. If the control population exhibits a type I survival curve (i.e. $c = 5.0$) and the cohort under the most intense level of treatment is expected to follow a type III pattern (i.e. $c = 1.0$), it is advantageous to allocate a greater number of individuals to the treatment factor at the expense of the control. Given the considerable investment in time and resources required to conduct life-table experiments, such preliminary explorations are certainly cost effective. These explorations might also suggest fractional replications of an experiment in which information on reproduction is obtained on some subset of the entire cohort. Thus, l_x could be based on say 80 animals and m_x information collected on only 20 animals.

Sensitivity analyses of Lotka's (1907a, 1907b) equation, beginning with the seminal work of Cole (1954) and followed by many others (e.g. Caswell 1978), have indicated the pronounced effect of variations in age at first reproduction on λ. We did not consider the age at first reproduction to be a random variable because we have generally found little variation within a given experimental treatment. In our experiments, the range of ages at first reproduction was almost always less than or equal to the egg-development period. This is precisely the same mechanism employed in our model. If the age at first reproduction varies significantly within a treatment, it may be indicative of lack of experimental control or undesirably high variation in the conditions of the neonates. One can reasonably expect variation in age at maturity among treatments (e.g. different ration levels or temperatures) but this variation is explicitly accounted for by the simulation method. Analysis of the effect of such variation on the sampling distribution of λ could be the subject of future research. Such research would be relevant to life-table experiments on organisms that exhibit intrinsically high variation, such as sexual populations in variable environments.

One source of variation that has not been addressed is the effect of parameter variability in the underlying survival model. If Weibull model parameters are estimated from actual data, these parameters are also random variables with specified distributions. Consequently, the p_x values in the Bernoulli trials become variables rather than constants. The effect of this additional source of variation would be to increase the variability of λ but it is not clear whether the magnitude of this increase would be significant. This could be another area of fruitful research.

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Appendix A. Derivation of Partial Derivatives of λ_N With Respect to $\bar{n}$ for K Lives

First, multiply Eq. 18 by ${\lambda_N}^A$ and rearrange to obtain

$$0 = {\lambda_N}^A - \sum_{x=1}^{A} {\lambda_N}^{(A-x)} \sum_{k=1}^{K} (n_k B_{x,k})/N. \quad \text{(A1.1)}$$

Now differentiate λ_N with respect to n_k:

$$0 = A{\lambda_N}^{(A-1)} \frac{\partial \lambda_N}{\partial n_k} - \sum_{x=1}^{A} (A-x){\lambda_N}^{(A-x-1)} \frac{\partial \lambda_N}{\partial n_k} \sum_{k=1}^{K} (n_k B_{x,k})/N - \sum_{x=1}^{A} ({\lambda_N}^{(A-x)} B_{x,k})/N. \quad \text{(A1.2)}$$

Solving Eq. A1.2 for $\partial\lambda_N/\partial n_k$ yields

$$\text{(A1.3)}\quad \frac{\partial\lambda_N}{\partial n_k} = \frac{\sum_{x=1}^{A} (\lambda_N{}^{(A-x)}B_{x,k})/N}{A\lambda_N{}^{(A-1)} - \sum_{x=1}^{A}(A-x)\lambda_N{}^{(A-x-1)}\sum_{k=1}^{K}(n_kB_{x,k})/N}.$$

These partial derivatives are evaluated at the sample estimates of λ_N and n_k, which are $\hat{\lambda}_N$ and $\hat{n}_k$, respectively, when they are included in the Taylor series variance estimator $s_T{}^2$.

Appendix B. Relation between K and N Distinct Lives in Taylor Series Approximation of the Variance of λ

If there are only K possible lives in a sample of size N but the observations are assumed to be distinct, then $n_i = 1$ for $i = 1, 2, \ldots, N$. The variance and covariance terms in Eq. 19 are equal to $(N-1)/N$ and $-1/N$, respectively. However, there will still only be K distinct values of D_i, i.e. the partial derivatives of Eq. 18 evaluated at the sample estimates of λ_N and $\bar{n}$. Define these distinct elements as $D_1, D_2, \ldots, D_K$ and let their respective frequencies be $n_1, n_2, \ldots, n_K$. Let A_i equal the partial derivative evaluated as in Eq. A1.3 where $n_k = 1$ and the summation over $k = 1, 2, \ldots, K$ is replaced by a summation over $i = 1, 2, \ldots, N$. Order the A_i such that

$$\text{(A2.1)}\quad A_1 = A_2 = \ldots = A_{n_1} = D_1;\quad A_{n_1+1} = A_{n_1+2} = \ldots = A_{n_1+n_2} = D_2;\ \text{etc.}$$

Eq. 19 thus becomes

$$\text{(A2.2)}\quad s_T{}^2 = \sum_{i=1}^{N} A_i{}^2(N-1)/N + \sum_{i\neq j=1}^{N}\sum^{N} A_iA_j(-1/N).$$

Not all of the $A_i{}^2$ and A_iA_j on the right-hand side of Eq. A2.2 are distinct. For each distinct D_k there are n_k cases in which $A_i{}^2 = D_k{}^2$ and $n_k(n_k-1)$ cross-product terms in which $A_i = A_j = D_k$. Collecting these terms together from Eq. A2.2 yields

$$\text{(A2.3)}\quad \sum_{k=1}^{K} n_kD_k{}^2(N-1)/N + \sum_{k=1}^{K} n_k(n_k-1)D_kD_k(-1/N) = \sum_{k=1}^{K} D_k{}^2n_k(N-n_k)/N$$

which is equal to the first term on the right-hand side of Eq. 19. There are now

$$\left(N^2 - \sum_{k=1}^{K} n_k{}^2\right)$$

cross-product terms in the second right-hand side term of Eq. A2.2 in which $A_i \neq A_j$. Following from Eq. A2.1, there will be $2n_1n_2$ pairings of D_1 with D_2, $2n_1n_3$ pairings of D_1 with D_3, and so on. By collecting these terms and substituting D_k for the appropriate A_i in Eq. A2.2, the second term becomes

$$\text{(A2.4)}\quad \sum_{i\neq j=1}^{K}\sum^{K} D_iD_j(-n_in_j/N).$$

Adding Eq. A2.3 to Eq. A2.4 yields Eq. 19, thereby completing the proof.

Influence of Chlorine on the Susceptibility of Striped Bass (*Morone saxatilis*) to *Vibrio anguillarum*

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Exposure of striped bass (*Morone saxatilis*) for 96 h to sublethal concentrations of total residual chlorine (TRC) (0.05–0.23 mg/L) did not increase their susceptibility to infection with the bacterial pathogen *Vibrio anguillarum*. Variables examined were TRC concentrations, length of exposure to chlorine, and the order of exposure to chlorine and the pathogen. Mortalities in the groups exposed to both chlorine and pathogen were not significantly different from those seen in groups receiving the bacteria only. The LD_{50} of *V. anguillarum* for striped bass was influenced by the water temperature as well as the size and immune status of the fish.

Une exposition de 96 h de bars rayés (*Morone saxatilis*) à des concentrations sublétales (0,05–0,23 mg/L) de chlore résiduel total (CRT) n'a pas accrû leur sensibilité à l'infection par la bactérie pathogène *Vibrio anguillarum*. La concentration de CRT, la durée d'exposition au chlore et l'ordre de l'exposition au chlore et au pathogène ont été les variables étudiées. La mortalité chez les groupes exposés au chlore et au pathogène ne différait pas de façon significative de celle notée chez les groupes exclusivement exposés à la bactérie. La DL_{50} de *V. anguillarum* pour le bar rayé était fonction de la température de l'eau ainsi que de la taille et de l'état immunitaire des poissons.

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In recent years, chemical pollutants have been increasingly implicated in fish disease outbreaks (Snieszko 1974; Sindermann 1979). While many fish kills have been directly traceable to the introduction of a variety of toxic pollutants, the subtle effects that low levels of pollutants have on fish populations are probably more important than the "large spill," since the effects are less likely to be obvious and the source more difficult to detect in time to save the environment. Documentation of these subtle effects has been difficult to obtain. However, in the last few years, laboratory studies have demonstrated altered susceptibility to pathogens or impaired immunological responses in fish exposed to pollutants, mostly heavy metals. Zinc, copper, and methylmercury have been reported to reduce the antibody response of two exotic fish species, zebrafish (*Brachydanio rerio*) (Sarot and Perlmutter 1976) and blue gourami (*Trichogaster trichopterus*) (Roales and Perlmutter 1977), to bacterial and viral antigens. Hetrick et al. (1979) found that young rainbow trout were more susceptible to infectious hematopoietic necrosis virus following exposure to sublethal copper levels. Increased susceptibilities of steelhead trout (*Salmo gairdneri*) to *Yersinia ruckeri* (Knittel 1981) and chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout

to *Vibrio anguillarum* (Baker et al. 1983) have also been noted following sublethal copper exposure. Sugatt (1980) reported reduced agglutinin titers to *V. anguillarum* in coho salmon (*Oncorhynchus kisutch*) following their exposure to chromium.

Chlorine is a biocide widely used for disinfection purposes by municipal sewage treatment plants and for biofouling control by power plants. Several investigators have shown that chlorine and its by-products can act directly on aquatic organisms to cause both lethal and sublethal effects (Brungs 1973, 1976; Hall et al. 1981), but none have investigated the effect that exposure to sublethal concentrations of chlorine might have on the susceptibility of aquatic organisms to disease. The striped bass (*Morone saxatilis*) is a recreationally and commercially important fish on both coasts of the United States (Scott and Crossman 1973). It has been suggested that the population decline of this valuable species is partially attributable to increased chlorine use (Coulter 1984). The focus of this study therefore was to determine if exposure of striped bass to sublethal concentrations of chlorine affected their susceptibility to bacterial infection. *Vibrio anguillarum* was chosen as the test pathogen because of its worldwide association with diseases of anadromous and catadromous fish (Anderson and Conroy 1970; Egidius and Andersen 1977; Fryer et al. 1972; Tajima et al. 1981).

Materials and Methods

Juvenile striped bass were obtained from freshwater hatcheries in Orangeburg, SC, Edenton, NC, Harrison Lake, VA, and Middletown, DE. The fish were acclimated to salt water for at least 2 wk prior to use They were maintained in 1893-L circular fiberglass tanks at a density of <0.1 g of fish/L and were fed trout chow daily. The salinity, pH, and dissolved oxygen range of the water was 9–13‰, 7.1–8.0, and 4.8–8.2 mg/L, respectively, during the study. The water temperature is given with the results of each experiment. All experiments were conducted at the Johns Hopkins University's Applied Physics Laboratory Facility in Shady Side, MD, using Chesapeake Bay water from Parish Creek.

Because of the need to obtain fish from different suppliers, the striped bass used were not of uniform size. For this reason, we determined LC_{50} values for each size striped bass using continuous-flow methods described previously (Hall et al. 1982). LC_{50} value and 95% confidence limits were calculated by using the logistic function as described by Ashton (1972). Ninety-six-hour LC_{50} values of 0.14, 0.19, and 0.23 mg/L total residual chlorine (TRC) were reported for 22-d larvae, 60-d juveniles, and 388-d juveniles, respectively (Hall et al. 1982). TRC concentrations were measured amperometrically (APHA 1981) using a Fisher and Porter titrator. TRC concentrations (0.05–0.23 mg/L) used in the stress experiments represented conditions that could be found at various industrial operations such as once-through cooling systems of power plant facilities (U.S. EPA 1982).

Vibrio anguillarum strain 775, obtained from Jorge Crosa, Oregon Health Sciences Center, Portland, OR, was the pathogen employed. This strain is highly virulent for salmonid species in the Pacific Northwest and the virulence is associated with plasmid carriage (Crosa et al. 1977). The organism was passed twice by intraperitoneal injection into juvenile striped bass prior to use. Isolates from moribund fish at the second passage level were propagated and stored at −70°C until needed. Bacteria were routinely grown in trypticase soy broth (Difco) with 1.5% NaCl at 20°C.

For preparation of inocula for fish, 24-h broth cultures were centrifuged and the bacterial pellets resuspended in sterile phosphate-buffered saline (PBS), pH 7.2. Optical density was measured at 540 nm (Spectronic 20) and organisms per millilitre derived from a standard curve. Appropriate dilutions of the suspension were made in PBS to obtain the desired concentration of bacterial cells. Actual numbers used in the inocula were verified by plate counts on trypticase soy agar (TSA) containing 1.5% NaCl.

A waterborne challenge with *V. anguillarum* did not give consistent results. Often, scattered deaths occurred over a range of bacterial doses and in those experiments where the deaths were dose-related, unrealistic numbers of bacteria (10^6 or 10^7 bacteria/mL) were needed to produce mortalities. Consequently, the intraperitoneal route of inoculation was used to ensure a reproducible challenge system. The fish were inoculated with 0.1 mL of a suspension containing the desired bacterial numbers. No anaesthetic was employed.

The following experimental conditions were employed: fish were exposed to sublethal doses of chlorine for 96 h prior to bacterial infection; fish were exposed to a single dose of chlorine for varying lengths of time prior to bacterial infection; and fish were inoculated with bacteria 24 h prior to chlorine exposure. Controls for each experiment consisted of untreated fish, fish inoculated with 0.1 mL of PBS, and fish receiving either chlorine treatment or bacteria only. Twenty fish were used for each experimental and control group. The data were examined for statistically significant differences by the log–rank test (Kalbfleisch and Prentice 1980).

The fish were examined daily for 14 d following challenge with *V. anguillarum*. Bacterial isolations were made from randomly selected moribund fish in each applicable experimental group. Fish were killed, sprayed with 70% ethanol, and aseptically opened. Samples from the kidney and liver were streaked onto both TSA with 1.5% NaCl and thiosulfate citrate bile salt sucrose (TCBS) agar (Oxoid). Plates were incubated at 20°C for 48 h and isolated colonies were subcultured. The isolates were presumptively identified as *V. anguillarum* if they proved to be gram-negative, motile roads that were sensitive to the vibriostatic agent 0/129 (Sigma) and novobiocin (Difco). Identification was confirmed by biochemical methods described by Shotts and Bullock (1975).

An enzyme-linked immunosorbent assay (ELISA) was used to monitor fish for antibody to *V. anguillarum* (Roberson 1981). The anti-bass globulin was prepared by immunizing rabbits with globulins precipitated from the serum of commercially caught striped bass. The peroxidase-labelled goat anti-rabbit globulin was obtained from Cappel Laboratories, Downington, PA.

Total bacteria and numbers of vibrios in the water supplying the holding tanks were estimated from samples taken twice each week. Water samples were plated in duplicate (0.1-mL inoculum) on both TSA with 1.5% NaCl and TCBS agar. Colonies were counted after a 48-h incubation at 20°C.

Results

During the course of this study, a number of infectivity titrations with *V. anguillarum* were carried out using the i.p. route of inoculation. Two conclusions can be drawn from these results (Table 1). First, smaller fish are more susceptible than

TABLE 1. Infectivity of *V. anguillarum* strain 775 for striped bass by i.p. inoculation

Experiment	Fish: Total length (cm)	Fish: Source	Water conditions: Temp. (°C)	Water conditions: Salinity (‰)	LD_{50}[a]
1	10–12	S. Carolina	19–20	9.5–10	$10^{5.8}$
2	10–12	S. Carolina	23–24	10–10.5	$10^{7.2}$
3	3–4	Delmarva	23–24	10–11	$10^{5.1}$
4	15–16	Delmarva	25–27	10–12	$10^{7.8}$
5	14–15	S. Carolina	21–24	10–11	$10^{6.6}$
6	4–6	Harrison Lake	21–24	10–11	$10^{5.5}$
7	14–16	S. Carolina	16–19	10–12	$10^{5.3}$
8	8–9	Delmarva	15–19	10–12	$10^{3.8}$
9	10–12	Delmarva	14–15	10–11	$10^{4.1}$
10	10–12	Delmarva	5–11	9–10	$10^{5.4}$
11	6–7	Delmarva	19–21	9–11	$10^{5.4}$
12	14	Edenton	9–12	12–13	$10^{5.5}$

[a]Number of bacteria that will kill 50% of fish inoculated.

TABLE 2. Seasonal influence on antibody titers to *V. anguillarum* in normal striped bass maintained in Chesapeake Bay waters.

Month	Water temp. (°C)	Antibody titer[a]: <8	8	16	32	64	128	256	512	No. of fish	Geometric mean titer
1981 Nov.	5–11	0	0	2	1	1	4	0	0	8	58.7
Dec.	4–7	0	0	4	6	2	0	0	0	12	28.4
1982 Mar.[b]	4–10	0	1	1	0	2	3	3	1	11	56.0
May	18–23	0	0	0	0	1	3	2	1	7	172.0
June	19–25	0	0	0	0	1	2	3	2	8	215.0
July	26–30	0	0	0	0	1	3	9	2	15	223.0
Aug.	22–27	0	0	0	0	5	4	4	0	13	121.0
Sept.	21–24	0	0	0	1	7	2	2	0	12	85
Oct.	11–22	0	0	1	1	0	2	1	0	5	74
Nov.	12–17	4	5	2	0	0	0	0	0	11	7
Dec.	7–13	4	1	0	0	0	0	0	0	5	4.6

[a]Reciprocal of the highest dilution of serum giving a positive response in the ELISA test.
[b]Fish obtained from commercial catch.

larger fish as illustrated by comparing the results of experiments 7 and 8. Second, the LD_{50} is markedly affected by the ambient water temperature in that fewer bacteria are needed to kill fish at lower temperatures. Substantially fewer organisms (<0.1%) were required to infect the same size fish at 14–15°C than at 23–24°C (experiments 2 and 9). Water temperature effects are also illustrated by the results of experiments 1 and 2 and by the large numbers of bacteria needed to infect fish at water temperatures of 25–27°C.

One contributing factor to this increased resistance of fish at higher water temperatures appears to be related to their immune status. As determined by ELISA, sera collected from uninoculated bass during the winter or spring were generally low in antibody titers to *V. anguillarum* (Table 2). However, by the summer months, the fish being held for experimental use had developed much higher antibody levels. This coincides with the significant increases in numbers of vibrio's (200–600 mL) appearing in May in the Chesapeake Bay water used to supply the holding tanks. Similar levels of *V. anguillarum* and related vibrios were present in the water through August after which they decreased in numbers as the water temperature dropped. The greater number of organisms needed to infect the fish at higher water temperatures might be due to an immune response gained from exposure to increasing numbers of *V. anguillarum* and cross-reacting vibrios in the water column. It is also well documented that the immune response of fish is enhanced at elevated water temperatures (see review by Corbel 1975).

One experiment that is at odds with this general observation is No. 10 (Table 1) in which greater numbers of bacteria were needed to infect 10- to 12-cm fish at 5–11°C than at 14–15°C (experiment 9). This may be explainable by the fact that *V. anguillarum* replicates more slowly at temperatures of 10°C or lower, which was the general range for experiment 10. In this regard, bacterial counts of the water supplying our facility showed that the numbers of *V. anguillarum* were considerably lower (0–80/mL) at temperatures below 10°C.

Exposure of Fish to Chlorine prior to Bacterial Infection

The initial study was designed to determine the effects of a

TABLE 3. Effect of exposure of striped bass to chlorine on their susceptibility to infection with *V. anguillarum* (fish size = 10–11 cm; source = South Carolina hatchery; water temperature = 19–20°C; ND = not done).

Bacterial dose[a] (i.p.)	Chlorine concentration (mg/L)[b]			
	0	0.05	0.10	0.20
$10^{6.2}$	20/20	ND	ND	ND
$10^{5.2}$	3/20	0/20	3/20	0/20
$10^{4.2}$	0/20	0/20	0/20	0/20
$10^{3.2}$	0/20	0/20	0/20	0/20
PBS	0/20	0/20	0/20	0/20
Control	0/20			

[a]Number of bacteria inoculated per fish.
[b]Fish were exposed to various chlorine levels for 96 h prior to bacterial challenge. Data = mortalities per number inoculated.

96-h TRC exposure (0.05, 0.10, and 0.20 mg/L) on the susceptibility of striped bass juveniles (10–11 cm) to *V. anguillarum*. If chlorine exposure was a predisposing factor, we would expect significantly more deaths in the chlorine-treated groups than in the fish receiving bacteria only. This clearly did not occur because there was no apparent difference between the chlorine-exposed groups and the bacterial controls (Table 3).

Two similar experiments were conducted. In the first, 96-h TRC (0.05, 0.08, and 0.11 mg/L) exposures of striped bass fingerlings were followed by infection with 10^5 or 10^6 bacteria. In the second, the effect of a 96-h TRC (0.20 mg/L) exposure on the susceptibility of fingerlings to doses of 10^4 to 10^7 organisms was evaluated. The results of these two studies (data not shown) were similar to those reported in Table 3 in that there was no evidence that exposure to chlorine at concentrations up to 0.2 mg/L resulted in increased mortalities of infected fish when compared with those groups that received the same bacterial doses only.

In the previous experiments, a standard 96-h exposure to chlorine was employed. It was possible that this was too long a treatment time and that the fish might have acclimated somewhat to the chlorine. To test this possibility, the effects of 6-, 12-, 24-, or 48-h exposures to 0.20 mg/L TRC prior to bacterial challenge were investigated. As in the previous experiments, no effect of the chlorine treatment was evident (Table 4).

Infection of Striped Bass prior to Chlorine Exposure

In this study, striped bass were infected with varying doses of *V. anguillarum* 24 h prior to being exposed to 0.225 mg/L TRC for 24 h. In addition to strain 775, we also employed a virulent strain (43 F) of *V. anguillarum* isolated from striped bass in the Chesapeake Bay (Toranzo et al. 1983). The 7-d mortality data with strain 775 show no difference between the untreated and chlorine-treated groups (Table 5). The 14-d data again show no difference between the $10^{5.9}$ and $10^{4.9}$ bacterial doses. At the $10^{3.9}$ dose, there was a 35% mortality in the chlorine-treated group compared with 5% mortality in the untreated group receiving the same dose. Since *V. anguillarum* was isolated from these fish, we do not feel that the deaths were from nonspecific causes such as other secondary bacterial invaders. With the 43 F strain, similar results were obtained with the chlorine-treated and untreated groups. Although with both vibrio strains the percentage mortalities after 14 d were usually higher in the chlorine-treated fish, the differences were not statistically significant nor were there differences in the distribution of survival times of treated and untreated groups.

TABLE 4. Effect of different times of exposure to chlorine on the susceptibility of striped bass to *V. anguillarum* (fish size = 8–9 cm; source = Delmarva hatchery; water temperature = 15–19°C; ND = not done).

Bacterial dose (i.p.)	Length of exposure to 0.20 mg/L chlorine (h)[a]				
	0	6	12	24	48
$10^{6.3}$	20/20	ND	ND	ND	ND
$10^{5.3}$	20/20	20/20	20/20	20/20	20/20
$10^{4.3}$	17/20	20/20	19/20	18/20	16/20
PBS	0/20	0/20	0/20	0/20	0/20
Control	0/20				

[a]Mortalities per number inoculated.

TABLE 5. Mortality of striped bass juveniles inoculated with two different strains of *V. anguillarum* followed by a 24-h exposure to 0.225 mg/L TRC (fish averaged 14.3 cm in length; water temperature = 9.5–13°C).

Vibrio strain	Dose[a]	Chlorine[b]	Days following chlorine exposure[c]			% mortality
			1	7	14	
775	$10^{5.9}$	+	0/20	18/20	19/20	95
		−	0/20	17/20	18/20	90
	$10^{4.9}$	+	0/20	4/20	14/20	70
		−	0/20	4/20	16/20	80
	$10^{3.9}$	+	0/20	0/20	7/20	35
		−	0/20	0/20	1/20	5
43F	$10^{5.2}$	+	0/20	14/20	15/20	75
		−	0/20	11/20	14/20	70
	$10^{4.2}$	+	0/20	3/20	9/20	45
		−	0/20	2/20	5/20	25
	$10^{3.2}$	+	0/20	0/20	4/20	20
		−	0/20	0/20	2/20	10
Controls	0	+	0/20	0/20	0/20	0
		−	0/20	0/20	0/20	0
	PBS	+	0/20	0/20	0/20	0
		−	0/20	0/20	0/20	0

[a]Number of bacteria inoculated (i.p.) per fish.
[b]+ denotes fish were exposed to chlorine for 24 h; − indicates no chlorine exposure.
[c]Mortalities per number of fish inoculated.

Discussion

Results of the bacterial infection studies indicate that the pathogenicity of *V. anguillarum* for striped bass is directly influenced by the size of the fish and the water temperature. Both of these factors affect the immune status of the host. Since *V. anguillarum* and related vibrios are present in Chesapeake Bay water, the longer the fish are held in such waters (i.e. the larger they become) the greater will be their immune response and the greater the bacterial dose needed to overcome the immunity. Similarly, higher water temperatures favor both a heightened immune response and increased numbers of *V. anguillarum* in the water. The ensuing immune response results

in the need for greater numbers of bacteria to produce a lethal infection. Our results with juvenile striped bass are similar to those reported by Watkins et al. (1981) with *V. anguillarum* in juvenile winter flounder (*Pseudopleuronectes americanus*) in terms of numbers of organisms per LD_{50} and the effect of water temperature on the infection.

Under the experimental conditions employed, we found no convincing evidence that exposing striped bass to sublethal chlorine levels increased their susceptibility to infection with *V. anguillarum*. This was true whether the fish were exposed to chlorine before or after bacterial challenge. Adequate control groups were employed and only scattered deaths occurred among the many fish used for this purpose. However, our results need to be qualified in terms of the limitations of the experimental conditions employed:

(1) By necessity, we had to obtain striped bass fingerlings from a variety of sources to obtain the numbers needed for this and other studies. Consequently, the fish were from genetically heterogenous stocks and how this influenced our results is unknown.

(2) We did not have the capability to heat or cool the ambient water to a constant temperature, which would have enabled us to control this important variable. The problems encountered when conducting experiments in excessively cold or warm water have been described above.

(3) Not infrequently, mortalities occurred when new fish were being transported or during their acclimation to Bay water. Consequently, only the hardiest fish survived for use in the chlorine stress studies.

(4) The development of higher antibody levels to *V. anguillarum* during the warmer months, when vibrio numbers increase in the water column, increased their resistance to the test pathogen.

Although our "real world" experimental design had these limitations, these variables also occur under natural conditions and could be important factors affecting the susceptibility of fish to disease. Fish do not live in water that has a constant temperature. They are genetically heterogenous in the wild. They are not protected from exposure to opportunistic pathogens. They do develop immunity to organisms present in the water and only the fittest survive to reproduce.

Conversely, our intraperitoneal challenge method was not a natural one but was necessitated by our inability to develop a successful water challenge. Similar findings have been reported with *V. anguillarum* by Tatner and Horne (1983) in which i.p. inoculation but not water challenge was successful in infecting rainbow trout fry. Nevertheless, in retrospect, it might have been worthwhile to attempt a water challenge of chlorine-exposed fish, since chlorine is known to cause damage to gill tissue and this mechanism of invasion may have been bypassed. Similarly, we did not address the possible chronic effects of chlorine, since a 96-h exposure time was the longest one tested. It is likely that striped bass would avoid most of the chlorine concentrations ($\geq$0.15 mg/L) used in this study during a 96-h period (Hall et al. 1983); therefore, longer exposures at these concentrations would not represent "real world" conditions. It is possible that prolonged exposures (>96 h) to low sublethal concentrations of chlorine might predispose striped bass to bacterial infections.

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NOTES

Comparisons of Growth in Weight of Temperate and Tropical Marine Fish Counterparts

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Edwards, R. R. C. 1984. Comparisons of growth in weight of temperate and tropical marine fish counterparts. Can. J. Fish. Aquat. Sci. 41: 1381–1384.

For temperate and tropical marine teleosts I compared growth in mean weight with age with similar trophic positions and ecological habits. Temperate demersal forms generally grew faster than tropical counterparts whereas mesopelagic and pelagic forms were similar. I suggest that differences in growth rates may be related to higher metabolic costs in the tropics in comparison with temperate waters.

L'auteur compare l'augmentation du poids moyen selon l'âge chez les téléostéens marins de niveaux trophiques et d'habitudes écologiques semblables vivant dans des régions tropicales et des régions tempérées. En général, les espèces démersales peuplant les zones tempérées grossissent plus vite que leurs cousines des zones tropicales, tandis que chez les formes mésopélagiques et pélagiques, la croissance était semblable. L'auteur élabore l'hypothèse que les différences entre les taux de croissance sont reliées à des coûts métaboliques plus élevés sous les tropiques par rapport aux eaux tempérées.

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In recent years, evidence has accumulated that tropical demersal fish stocks have low fisheries yields and are easily overexploited (Munro et al. 1971; Crowley 1983). Therefore, I decided to compare growth rates from the published literature for temperate and torpical fish counterparts from similar ecological niches.

Although conventional wisdom states that tropical fish grow faster than temperate forms, actual comparisons of growth in weight between these two groups of fish have rarely been conducted. In fact, Pauly and Martosubroto (1980) showed that the tropical threadfin bream (*Nemipterus marginatus*) is very slow growing. Edwards et al. (1971) concluded that tropical soles (*Cynoglossus* spp.) grew slower than temperate flatfish and showed marked metabolic compensation for temperature. Comparisons of growth in length using the von Bertalanfty K constant are influenced by the lower average L (infinity) in the tropics producing higher K values as shown for Scombridae by Pauly (1980). For this reason I made a comparison using growth in length converted to growth in weight from published weight to length relationships. I selected counterpart species from the published literature using several criteria, including observations on composition of the diet and changes with the age of the fish. Some of these are my own unpublished observations, whereas others are from the literature cited here. I also studied the literature on tropical fisheries; using my own experience, in part, I categorized the fish as demersal, mesopelagic, or pelagic. Finally I chose only species that are abundant in commercial catches from their respective localities and avoided less common and more specialized forms. The species selected from the literature are listed in Table 1; the mean weights are given as 7 yr for demersal fish and 3 yr for demersal fish and 3 yr for the shorter lived pelagic fish. In Fig. 1 are a selection of growth curves I prepared for this analysis. Tropical demersal fish tend to have slower growth rates than temperate counterparts whereas the mesopelagic and pelagic forms are similar. The tropical piscivorous Scombridae (e.g. Spanish mackerels, tunas) have very high growth rates (Shomura and Keala 1962; Devaraj 1979; Pauly 1979a) but have not been included here because there are no suitable counterparts.

Possible explanations for retarded growth rates in the tropics are as follows:

(1) Maintenance metabolism on a unit weight basis is higher in the tropics (Edwards et al. 1970; Pauly 1979a).

(2) Metabolic costs for reproduction are higher in the tropics. Evidence for early maturation, prolonged serial spawning, and higher comparative fecundities exists (Matsumoto 1966; Luther 1973; Guerra and Bashirullah 1975).

(3) Available food sources may be more limiting in the tropics. Krey (1973) showed that tropical waters not affected by upwelling were mainly oligotrophic, whereas Edwards (1973) found lower standing crops of benthos below the thermocline in the Caribbean than occurred in comparable temperate environ-

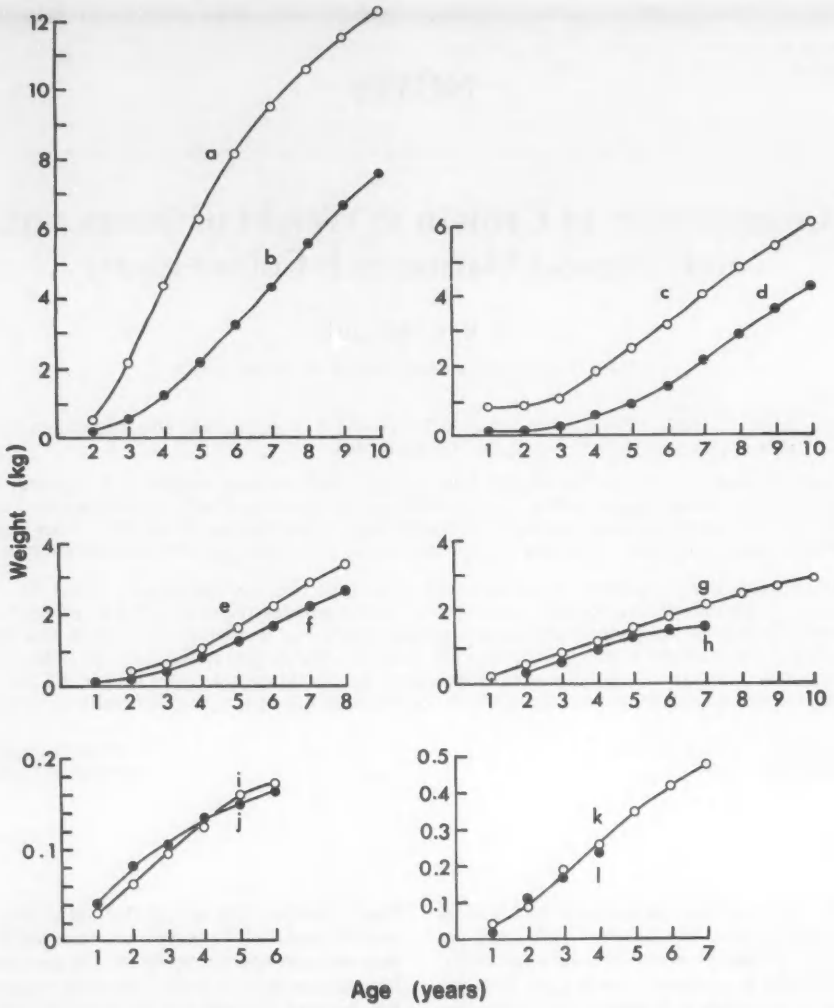

FIG. 1. Mean growth in fresh weight of tropical (●) and temperate (○) marine teleost fish counterparts. (a) *Gadus morhua* (Daan 1974); (b) *Lutjanus sanguineus* (Lai and Liu 1974); (c) *Pollachius virens* (Jones and Jonsson 1971); (d) *Lutjanus purpureus* (Fonteles-Filho 1970); (e) *Gadus pollachius* (Moreau 1964); (f) *Lutjanus campechanus* (Bradley and Ryan 1975); (g) *Melanogrammus aeglefinus* (Jones 1962); (h) *Pseudolithus typus* (Longhurst 1964); (i) *Micromesistius poutassou* (Bailey 1982); (j) *Nemipterus marginatus* (Pauly and Martosubroto 1980); (k) *Scomber scombrus* (Lockwood and Johnson 1976); (l) *Rastrelliger Kanagurta* (George and Banerji 1964).

ments. Evidence to support limitation by food sources may be inferred from Lai and Liu (1974), Su and Liu (1975), and Yeh et al. (1977). These authors found that growth rates of demersal fish in the South China Sea were considerably less than for the same species in the East China Sea. Geographical differences in growth rates occur in temperate waters (Daan 1974); Rae (1965) attributed these to differences in quantity and quality of food sources.

A combination of these factors may result in reduced growth rates of demersal fish in the tropics. Tropical fish mature early at 2–3 yr but no abrupt reduction in growth rate appears to occur at this stage (Lai and Liu 1974). Pauly (1979a) suggested that gill surface area possibly limits oxygen uptake and this thus limits active metabolism in tropical fish inhabiting waters with reduced oxygen contents.

The higher fecundities in the tropics support the observations of Pauly (1980) of higher natural mortality rates. Stevenson (1977) observed slow growth rates but high mortality rates for Carangidae (jacks) in the Caribbean. There is a scarcity of information on growth and mortality rates in tropical multi-species fish stocks for formulation of management policies of the types discussed by Pauly (1979b) for the Gulf of Thailand, where standing stocks were lower than in temperate waters. Pauly (1979b) indicated a rapid decline in catch per unit effort

TABLE 1. Calculated mean live weights attained by temperate and tropical marine fish counterparts (weights obtained from length at age and weight–length relationships).

Temperate		Tropical	
Species	Wt (kg)	Species	Wt (kg)
Demersal flatfish (age 7 yr)			
Pleuronectes platessa (Bowers and Lee 1971)	0.70	*Cynoglossus macrolepidotus* (Krishnan Kutty 1967)	0.14
Solea solea (De Veen 1976)	0.60	*Cynoglossus bilineatus* (Seshappa 1978)	0.16
Demersal roundfish (age 7 yr)			
Gadus morhua (Daan 1974)	9.0	*Lutjanus sanguineus* (Lai and Liu 1974)	2.80
Pollachius virens (Jones and Jonsson 1971)	10.0	*Lutjanus purpureus* (Fonteles-Filho 1970)	5.73
Gadus pollachius (Moreau 1964)	2.89	*Lutjanus campechanus* (Bradley and Ryan 1975)	2.50
Melanogramus aeglefins (Jones 1962)	1.80	*Pseudolithus typus* (Longhurst 1964)	1.50
Chrysophrys unicolor (Paul and Tarring 1980)	1.51	*Lethrinus enigmaticus* (Lebau and Cueff 1975)	0.76
Mesopelagic roundfish (age 3 yr)			
Micromesistius poutassou (Bailey 1982)	0.12	*Nemipterus marginatus* (Pauly and Martosubroto 1980)	0.09
Pelagic roundfish (age 3 yr)			
Scomber scombrus (Lockwood and Johnson 1976)	0.17	*Rastrelliger kanagurta* (George and Banerji 1964)	0.16
Clupea harengus (Anonymous 1981)	0.20	*Sardinella longiceps* (Antony Raja 1972)	0.06

during expansion of trawling activities; Pauly (1979b) and Edwards (1983) showed large changes in species composition for tropical fish stocks under exploitation.

I concluded that the biomass of tropical demersal fish stocks has a lower intrinsic growth potential than that in temperate waters. The application of fisheries yield models in the tropics should therefore take this into consideration.

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Evaluation of Acid-Insoluble Ash as an Indicator of Feed Digestibility in Rainbow Trout (*Salmo gairdneri*)

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Atkinson, J. L., J. W. Hilton, and S. J. Slinger. 1984. Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 41: 1384–1386.

Relative performances of dietary acid-insoluble ash, celite (a source of acid-insoluble ash), and chromic oxide as digestibility references were compared. Apparent digestibilities of dry matter, crude protein, and gross energy in a practical diet fed to rainbow trout (*Salmo gairdneri*) were similar regardless of indicator used. Acid-insoluble ash can be an effective indicator for digestibility trials with fish. Its natural occurrence in fish foods and feedstuffs and ease of analysis make it preferable to added indicators, such as chromic oxide, in many circumstances. When the acid-insoluble ash content of a diet is low, the addition of celite can improve the precision of the analysis without affecting absolute values of digestibility coefficients.

On compare les performance relatives de la cendre insoluble dans l'acide, de la célite (une source de cendre insoluble dans l'acide) et de l'oxyde chromique comme témoins de la digestibilité des aliments. Les digestibilités apparentes de la matière sèche, des protéines brutes et de l'énergie totale d'une ration donnée à des truites arc-en-ciel (*Salmo gairdneri*) étaient semblables indépendamment de l'indicateur utilisé. La cendre insoluble dans l'acide peut être un indicateur efficace pour des essais de digestibilité chez les poissons. Sa présence naturelle dans les aliments du poisson et des animaux domestiques et sa facilité d'analyse la rend souvent préférable aux indicateurs ajoutés, comme l'oxyde chromique. Quand la teneur en cendre insoluble dans l'acide d'un régime est faible, l'addition de célite peut améliorer la précision de l'analyse sans influer sur les valeurs absolues des coefficients de digestibilité.

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Determination of digestive efficiency requires either collection of the total fecal matter produced after consumption of a known amount of feed or measurement of the concentration of an inert indicator in the feed and feces. Complete collection of fish fecal matter is at best tedious and in many circumstances impossible. Therefore, the indicator method of determining digestibility coefficients has been preferred.

To be an effective indicator, a substance must be indigestible and unabsorbable, remain homogeneously mixed with the

digesta during passage through the gut, and have no effect on the digestive metabolism of the animal (Schneider and Flatt 1975). Two classes of indicators can be distinguished: those that are added to a diet and those that are inherently present as natural components of a diet. Chromic oxide (Cr_2O_3) is the substance most commonly added as an indicator in digestibility studies with fish. However, this method is generally restricted to specially formulated semipurified or practical diets. In commercial fish feeds or natural fish diets, such as algae or zooplankton, addition of Cr_2O_3 is usually not possible. Furthermore, even if the Cr_2O_3 could be mixed with a natural food, the acceptability of such diets to fish has been questioned (Bowen 1978). The use of indicators that are inherent components of fish feeds or natural foods avoids these problems, and several potential reference compounds have been investigated.

Buddington (1980) recommended the use of hydrolysis-resistant organic matter (HROM), primarily composed of cellulose and chitin. However, several species of fish have been reported to possess either cellulase (Stickney 1979) or chitinase activity (Danulat and Kausch 1984), and although these may not affect HROM in specific situations (Buddington 1980), the general assumption that this fraction of the diet is indigestible is questionable. The use of crude fiber as an indicator (De Silva and Perera 1983) can be criticized on similar grounds. The insoluble mineral component of fish diets, whether determined as silica (Hickling 1966), hydrolysis-resistant ash (Bowen 1981), or acid-insoluble ash (AIA), shows potential as an indicator.

Research in ruminants (Van Keulen and Young 1977), pigs (McCarthy et al. 1974), and poultry (Vogtmann et al. 1975) has validated the use of AIA, which is primarily silica, as a naturally occurring marker for digestibility trials. The purpose of this study was to compare and evaluate AIA and Cr_2O_3 as digestibility indicators in a practical diet for rainbow trout (*Salmo gairdneri*).

Materials and Methods

A practical diet was formulated containing 35% capelin meal, 25% soybean meal, 27% wheat middlings, 10% fish oil, and 2 and 1%, respectively, of vitamin and mineral premixes (Hilton and Slinger 1981). Two other diets were prepared by adding either celite (Johns-Mansville Ltd.), a form of diatomaceous silica, at a level of 1% of the diet or Cr_2O_3 (Fisher Scientific Ltd.) at a level of 0.5% of the diet.

The three test diets were processed by steam pelleting in a laboratory pellet mill and dried for 24 h at room temperature in a fan-ventilated chamber. Each test diet was fed to three groups of approximately 40 juvenile rainbow trout (initial weight ~ 100 g/fish) reared in a digestibility system consisting of three fiberglass tanks (45-L capacity), with a common drain to a feces settling column as described by Cho et al. (1982). The tanks had a water flow rate of 2–3 L/min of dechlorinated City of Guelph municipal water and were individually aerated and thermostatically maintained at 15°C. The trout were fed the test diets to satiety three times a day, as described by Hilton and Slinger (1981), for 8 d. The first 3 d constituted an acclimation period and no feces were collected during this time. During the next 5 d, daily feeding and feces collection cycles were carried out as described by Cho et al. (1982). Each of the five daily feces collections was immediately centrifuged at 10 000 × *g* for 20 min at 5°C. The supernatant was then discarded and the feces freeze-dried, ground, and stored in a refrigerator at −5°C until required for analysis.

Dry matter, crude protein, ash, and gross energy were analyzed as described in Horowitz (1980). Triplicate analyses of the practical diet gave mean values of 43.2% crude protein, 6.8% ash, and 5.38 kcal gross energy/g (22.52 kJ/g) expressed on a dry matter basis. The chromium content of samples containing Cr_2O_3 was determined by atomic absorption spectrophotometry (Arthur 1970). In the diet with Cr_2O_3 added, the analyzed value was 0.49% of the dry matter.

The AIA content of the practical diet, the diet with added celite, and the associated fecal samples was determined essentially by the method of Van Keulen and Young (1977). Samples of dried feed (10–12 g) or feces (3–4 g) were ashed for 16 h at 600°C. This is higher than the 450°C specified by Van Keulen and Young (1977) but was found to be necessary to ensure complete combustion of the organic material in the samples. The resulting ash was boiled in 100 mL of HCl (2 mol/L) for 5 min, filtered hot through ashless filter paper, and the residue washed with boiling water. Ashing the filter paper and residue for 16 h at 600°C gave the AIA; this was 0.29 and 1.33% of dry matter for the practical diet and diet with added celite, respectively.

In a preliminary study, the method for AIA determination described by Vogtmann et al. (1975) was found to be inappropriate for fish diet analysis. This involves direct digestion of feed and feces samples with more concentrated HCl (4 mol/L) followed by ashing of the filtered residue. The typically high fat content of practical trout diets precludes effective digestion, leading to poor filtration and a high degree of variability in the AIA value for feed samples. Ashing the samples prior to acid digestion as described by Van Keulen and Young (1977) eliminated this problem.

The apparent digestibility coefficients (DC) for dry matter, crude protein, and gross energy were calculated for the three test diets by the following formula (Schneider and Flatt 1975):

$$DC = 100 - 100 \times \frac{\%\ \text{indicator in feed}}{\%\ \text{indicator in feces}} \times \frac{\%\ \text{nutrient in feces}}{\%\ \text{nutrient in feed}}.$$

The results were then subjected to analysis of variance and compared using Tukey's honestly significant difference procedure (Steel and Torrie 1980).

Results and Discussion

A comparison of the apparent digestibility coefficients for dry matter, crude protein, and gross energy showed no significant difference ($P < 0.01$) between the three diets (Table 1), although the value using Cr_2O_3 as an indicator tended to be slightly lower than with AIA. Considering the similarity of these results and the relative simplicity of AIA analysis compared with the determination of chromium, the use of AIA as an indicator should be seriously considered for routine fish feed digestibility studies using diets formulated from practical ingredients. In addition, these results support the use of AIA as a digestibility indicator for natural diets for fish (Bowen 1981). However, this method may be compromised by very low or variable levels of insoluble ash in some natural diets, since De Silva and Perera (1983) found hydrolysis-resistant ash to give the most variable measure of the digestibility of protein in

TABLE 1. Apparent digestibility coefficients of the dry matter, crude protein, and gross energy content of the test diets (results based on the mean of five samples ± standard deviation).

Test diet	% digestibility		
	Dry matter	Crude protein	Gross energy
Practical diet	76.2±0.9	94.8±0.2	82.7±1.0
+ Celite	75.5±0.2	95.8±0.2	83.0±0.5
+ Cr_2O_3	73.8±2.0	94.1±0.6	81.2±1.4

Hydrilla fed to a cichlid when compared with HROM or crude fibre as a naturally occurring indicator.

Although the addition of celite was effective in raising the AIA level in the diet, it had no effect on the absolute values of the apparent digestibility coefficients determined. Nevertheless, as Thonney (1981) has reported, the addition of an exogenous AIA source can increase the precision of AIA measurement. This was substantiated in the present study by lower coefficients of variation for levels of AIA in diets with added celite (1.6%) relative to the unaltered practical diet (4.5%). When the endogenous AIA content of a diet is too low for precise measurement, the addition of AIA as celite, or some other form such as pumice, could be used as an easily measured and relatively inexpensive alternative to Cr_2O_3.

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Growth and Gonadal Development in Triploid Landlocked Atlantic Salmon (*Salmo salar*)[1]

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Benfey, T. J., and A. M. Sutterlin. 1984. Growth and gonadal development in triploid landlocked Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 41: 1387–1392.

There was no difference in weight between diploid and triploid landlocked Atlantic salmon (*Salmo salar*) measured over a 9-mo period, but triploids were consistently longer (FL) and thus had a lower condition factor than diploids. The gonadosomatic index (GSI) of triploid females was only 7.7% that of diploid females, while the GSI of triploid males was 52% that of diploid males. Triploid ovaries had the external appearance of undeveloped gonads, but every triploid female did in fact produce a small number of oocytes (from 1 to 12, versus several hundred oocytes in each diploid female). Triploid testes were well developed but contained few spermatids and no spermatozoa. Diploid testes, on the other hand, were in advanced stages of spermiogenesis. None of the triploid males reached spermiation.

Aucune différence de poids n'a été décelée chez des saumons atlantiques de l'intérieur (*Salmo salar*), diploïdes ou triploïdes, dont la taille a été mesurée pendant 9 mo ; les triploïdes étaient cependant constamment plus longs (longueur à la fouche) et présentaient donc un coefficient de condition moins élevé que les diploïdes. L'indice gonadosomatique (IGS) des femelles triploïdes ne s'élevait qu'à 7,7 % de celui des femelles diploïdes tandis que celui des mâles triploïdes représentait 52 % de celui des mâles diploïdes. Les ovaires des triploïdes ressemblaient à des gonades non développées, mais chaque femelle triploïde avait produit un petit nombre d'oocytes (de 1 à 12 par rapport à plusieurs centaines chez la femelle diploïde). Les testicules des triploïdes étaient bien développés mais contenaient peu de spermatides et aucun spermatozoïde. Par contre, les testicules des diploïdes étaient aux derniers stades de la spermiogenèse. Aucun des mâles triploïdes n'avait atteint le stade de la spermiation.

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The induction of triploidy in fish and its application to fish culture has recently been reviewed (Purdom 1983; Thorgaard 1983). The benefit of triploids to fish culture lies in their potential sterility, a consequence of having an uneven number of chromosome sets. Sterile fish are expected to avoid the deleterious side-effects of maturation, such as reduced appetite, reduced food conversion efficiency, deterioration of flesh quality, and high postspawning mortality. Triploid fish, particularly females, have a reduced gonadosomatic index (GSI) in comparison with diploids (Purdom 1976; Thorgaard and Gall 1979; Gervai et al. 1980; Lincoln 1981a, 1981b; Ueno and Arimoto 1982; Wolters et al. 1982), and gametogenesis does not generally proceed beyond the oogonial or spermatogonial stages (Purdom 1972; Gervai et al. 1980; Lincoln 1981a, 1981b; Wolters et al. 1982; Lincoln and Scott 1983; Yamazaki 1983). An apparent exception is the plaice, *Pleuronectes platessa*, in which triploid males have a higher GSI than diploids and produce functional, though apparently aneuploid, spermatozoa (Lincoln 1981a).

In juvenile fish of the same age, diploid and triploid individuals are generally the same size (Swarup 1959; Cuellar and Uyeno 1972; Purdom 1972; Gold and Avise 1976; Gervai et al. 1980). A possible exception is the blue tilapia, *Tilapia aurea*, in which polyploid individuals of uncertain ploidy were significantly larger than diploids at 14 wk (Valenti 1975). Adult triploids are generally heavier than diploids by the time that the diploids have spawned (Purdom 1976; Thorgaard and Gall 1979; Lincoln 1981c; Wolters et al. 1982). However, in hybrids between the plaice, *P. platessa*, and flounder, *Platichthys flesus*, diploid females have an accelerated postspawning growth rate and are the same size as triploids by 2 mo after spawning (Lincoln 1981c).

The various salmonid species cultured in Canada represent the major source of farmed fish production in this country (Anonymous 1983). A problem of concern in salmon farming is the onset of maturity at a small size or early age (Naevdal 1983), and triploids may therefore be particularly well suited for salmonid aquaculture. Conclusive data on the growth rates of triploid salmonids are lacking, but it has been suggested that they grow at slower rates than diploids (Thorgaard and Gall 1979; Refstie 1981; Thorgaard et al. 1982; Utter et al. 1983). If this is in fact the case, their benefit to fish farming may be reduced. The purpose of our research was therefore to examine growth and gonadal development in triploid Atlantic salmon (*Salmo salar*) to evaluate their suitability for commercial aquaculture. We chose to work with a dwarf, landlocked form of salmon in which diploids mature at an early age (Sutterlin and

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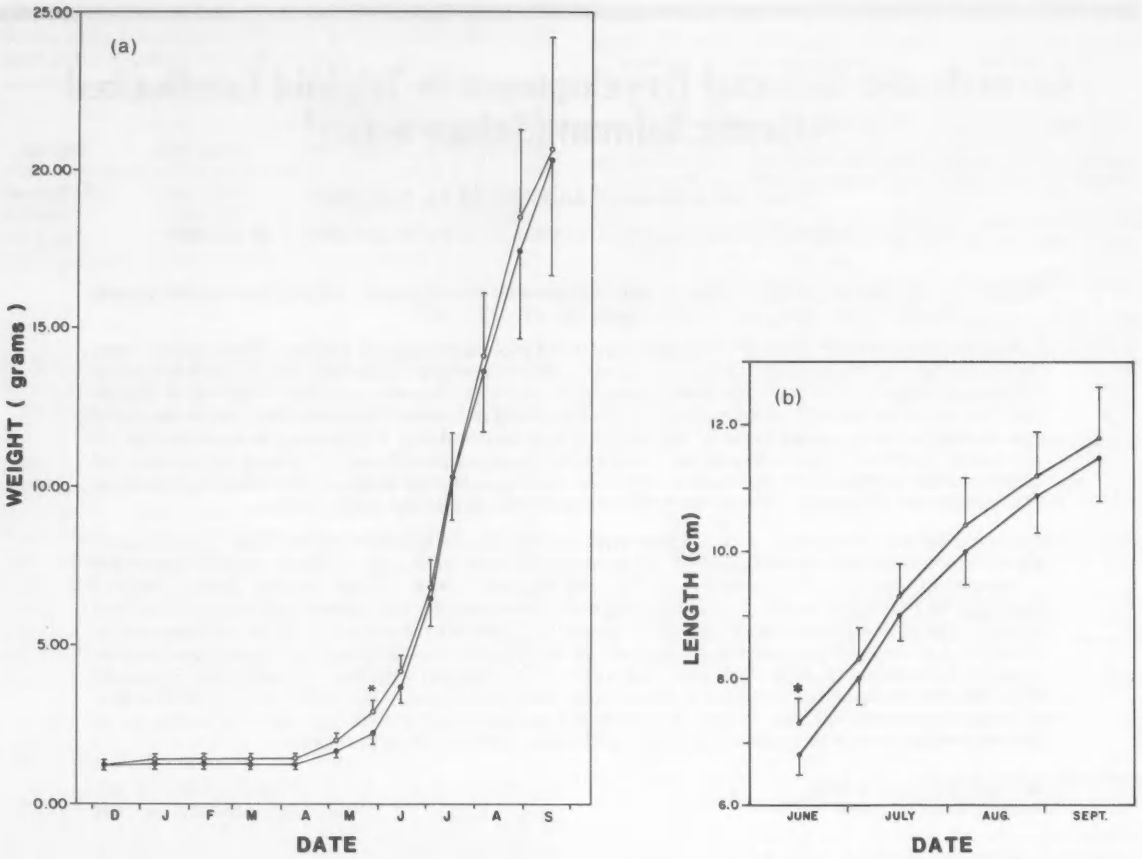

FIG. 1. Growth in (a) weight and (b) length of diploid (●) and triploid (○) Atlantic salmon (mean ± 95% confidence limits; $*P < 0.05$).

MacLean 1984), a trait we considered useful in examining subsequent events related to maturation in triploid salmonids.

Materials and Methods

Triploidy was induced by heat shocking eggs shortly after fertilization, thereby inhibiting extrusion of the second polar body (Benfey and Sutterlin 1984). Diploid and triploid individuals were identified at 9 mo after hatching by using a Coulter Counter and Channelyzer to measure median erythrocyte volume (Benfey et al. 1984). At this time, 39 diploid and 39 triploid fish were placed in adjacent 100 × 100 × 42 cm self-cleaning fiberglass tanks receiving a constant flow of oxygen-saturated water at ambient temperatures. Temperatures ranged from 1–2°C in December and January to 17–20°C in July and August. Approximate natural daylengths were simulated using artificial overhead lighting. Both groups were simultaneously fed excess rations of a moist pellet diet 2 to 3 times a day in the winter and 3 to 4 times a day in the summer.

Each fish was weighed (W) at 13 intervals over a 9-mo period spanning one growth season. In the final 6 intervals, fork length (FL) was also recorded and condition factor calculated as $CF = (W/FL^3) \times 100$. At the end of the growth experiment, 10 diploid and 10 triploid fish were killed (5 males and 5 females of each ploidy) and their wet weight determined. The gonads were then excised and weighed and the gonadosomatic index (GSI) calculated as

$$GSI = \frac{(\text{gonad weight}) \times 100\%}{(\text{total weight} - \text{gonad weight})}.$$

The gonads were then fixed for a minimum of 24 h in Bouin's fixative and prepared for histology by standard techniques (Humason 1972), with sections cut at 7 μm and stained in Gill's haematoxylin and eosin.

Single-factor analysis of variance was used to determine the level of significance for any differences encountered in weight, length, condition factor, or GSI between diploids and triploids. Identification of cell types in the gonads was based on the terminology used by Nagahama (1983).

Results

None of the fish grew over the first 5 intervals (Fig. 1a), which coincided with the lowest water temperatures during the winter. Triploids had a higher initial growth rate in the spring, and were significantly heavier than diploids by May 29. However, the growth rate of diploids soon increased and remained similar to that of the triploid fish for the duration of the experiment. Triploids were consistently longer (FL) than

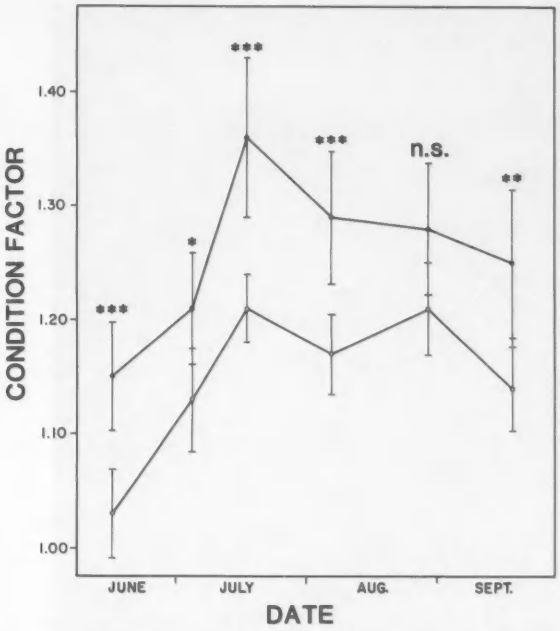

FIG. 2. Condition factor of diploid (●) and triploid (○) Atlantic salmon (mean ± 95% confidence limits; $***P < 0.001$, $**P < 0.005$, $*P < 0.05$, n.s. = not significant).

diploids (Fig. 1b) and thus had a lower condition factor (Fig. 2). Condition factor for both groups reached a maximum in mid-July, corresponding to the period of greatest growth, and began to fall towards the end of the summer.

Triploid ovaries were easily distinguished from those of diploid fish because of their smaller size. The GSI of triploid females was only 7.7% that of diploids (0.024 ± 0.007% SD versus 0.31 ± 0.04%, $P < 0.001$). Diploid ovaries were packed with hundreds of oocytes, mostly at previtellogenic stages with some advanced as far as endogenous vitellogenesis, whereas triploid ovaries were composed mostly of oogonia (Fig. 3). However, each triploid female possessed a few (from 1 to 12) previtellogenic oocytes indistinguishable from those encountered in diploid ovaries.

Triploid testes, although well developed, did not have the smooth surface typical of diploid testes and appeared to be more heavily vascularized. The GSI of triploid males was 52% that of diploids (3.0 ± 0.4% SD versus 5.8 ± 2.0%, $P < 0.025$). Diploid testes were in advanced stages of spermiogenesis and contained large regions of spermatids and spermatozoa, whereas triploid testes contained few cells developed beyond the spermatocyte stage (Fig. 4). No spermatozoa were observed histologically in triploid testes, and repeated attempts to strip triploid males were unsuccessful, even after all the diploids had spermiated.

Discussion

Growth rates are virtually identical for immature diploid and triploid Atlantic salmon. Similar results have been reported for other teleosts (Purdom 1976; Gervai et al. 1980; Lincoln 1981c; Wolters et al. 1982). It is not surprising that growth rates are the same for diploids and triploids prior to maturation, since little energy is diverted to gonadal development in either group. Triploids are expected to continue growing over the period when maturing diploids exhibit reduced or no growth. It was not possible to carry on the growth experiment with Atlantic salmon long enough to confirm this, but this is certainly the case in female triploid plaice × flounder hybrids (Purdom 1976; Lincoln 1981c) and channel catfish (*Ictalurus punctatus*) (Wolters et al. 1982).

The influence of triploidy on condition factor in Atlantic salmon closely resembles the situation in flatfish (Lincoln 1981c): in both cases, diploids exhibit an elevated condition factor prior to spawning. The data obtained by Lincoln are easier to interpret than those described here, since he used only female fish. The effect of triploidy on gonadal development was far greater in female flatfish than in males (Lincoln 1981a, 1981b), and it was therefore expected that differences in condition factor would be more pronounced in the females. Furthermore, the female salmon described in this paper were still 12 mo from spawning, and it is not clear what effect further growth might have on the condition factor of these fish.

In external appearance the ovaries of triploid Atlantic salmon resemble the undeveloped gonads of juvenile salmonids, as is the case in sterile triploid rainbow trout (*Salmo gairdneri*) (Thorgaard and Gall 1979; Lincoln and Scott 1983; Yamazaki 1983), but triploid testes are well developed. Such sexual dimorphism in gonadal development has previously been described in triploid fish (Purdom 1972; Thorgaard and Gall 1979; Gervai et al. 1980; Lincoln 1981a, 1981b; Wolters et al. 1982) and amphibians (Fankhauser 1940, 1941; Kawamura 1951a, 1951b) and is likely due to testes being at a much larger size than ovaries when their cells enter meiosis, at which point triploid gametogenesis is presumably disrupted. Meiosis in the teleost ovary begins when oogonia are transformed into primary oocytes and in the testis when primary spermatocytes become secondary spermatocytes (Nagahama 1983), the latter process occurring later in the life of salmonids (Nakamura 1982, and citations therein).

It is apparent that a small number of cells succeed in passing through the first meiotic division in both the ovaries and testes of triploid animals; female triploid rainbow trout are the only reported exception (Lincoln and Scott 1983; Yamazaki 1983). Nothing is known about the meiotic mechanism whereby postmeiotic cells are produced in such triploids, but it may be simply through the random segregation of chromosomes. This is supported by the fact that triploid amphibians generally produce aneuploid gametes (Fankhauser and Humphrey 1950, 1954; Kawamura 1951a, 1951b). However, functional meiotic mechanisms have evolved in gynogenetic triploids, involving either the formation of tripolar spindles (Cherfas 1969) or the endomitotic duplication of chromosomes prior to normal meiotic division (Cimino 1972; Cuellar 1976).

The reduction in gonadal development observed in triploid Atlantic salmon may be of benefit to aquaculture, as somatic growth is expected to be enhanced. It is clear that the effect of triploidy on gonadal development is far greater in females than in males. In commercial salmonid farming it is generally the males that mature at a smaller size and earlier age, and the most likely application of triploidy to fish culture may therefore be in conjunction with treatments producing all-female populations, as suggested by Lincoln and Scott (1983). In Atlantic salmon, the use of homogametic milt from sex-reversed females (Johnstone and Youngson 1984) in conjunction with heat or hydrostatic pressure shocks to inhibit polar body extrusion

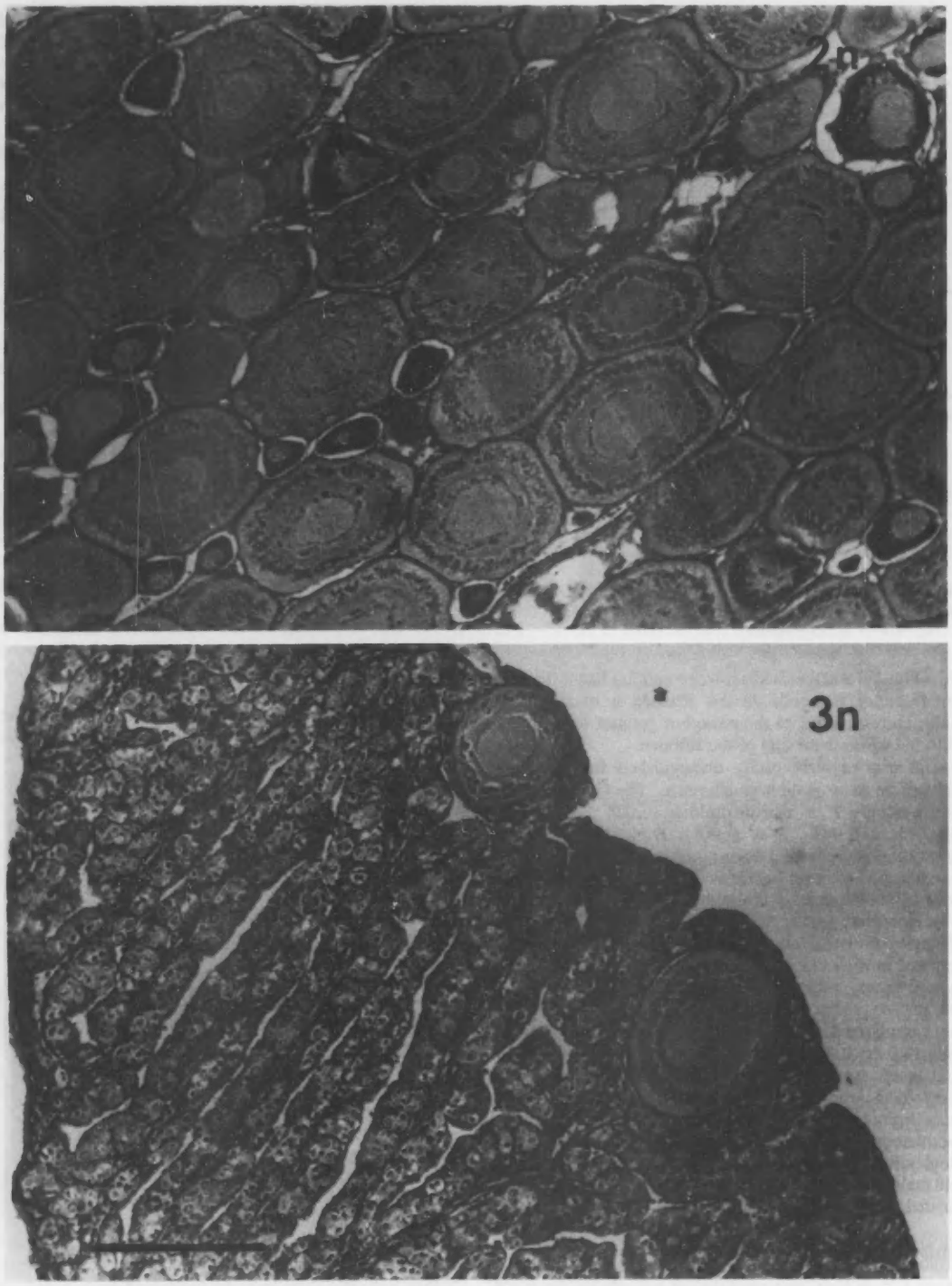

FIG. 3. Transverse sections of diploid ($2n$) and triploid ($3n$) ovaries (bar = 300 μm).

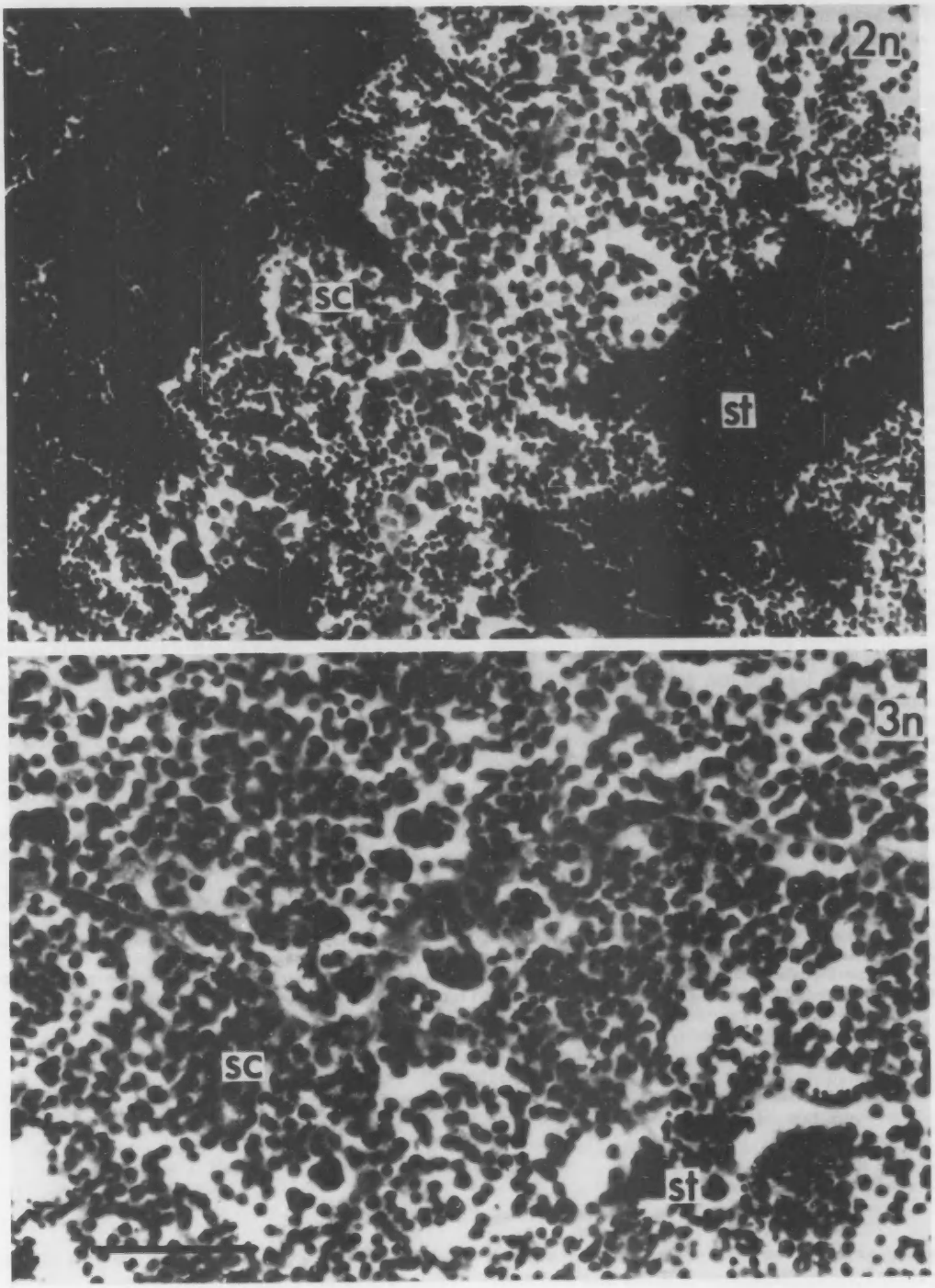

FIG. 4. Transverse sections of diploid ($2n$) and triploid ($3n$) testes, showing spermatocytes (sc) and spermatids (st) (bar = 75 μm).

(Benfey and Sutterlin 1984) should allow the effective production of all-female triploids.

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PERSPECTIVES

Multiattribute Analysis and the Concept of Optimum Yield

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Healey, M. C. 1984. Multiattribute analysis and the concept of optimum yield. Can. J. Fish. Aquat. Sci. 41: 1393–1406.

This paper reviews the origin and operational definition of the optimum yield (OY) concept and demonstrates how techniques of decision analysis can provide an analytical model for OY. The concept of OY was formalized as the guiding principle of fisheries management in the United States and Canada in 1976. The policies of both countries make it clear that a wide range of biological, economic, and social factors are to be taken into account in determining OY. Confusion exists, however, about precisely which of these factors should determine OY in any fishery and what is their relative importance. Uncertainty also exists about how to take biological, economic, and social factors jointly into account as the concept of OY implies one must. Established biological and economic models in fisheries are not adequate for such an analysis because their focus is single- rather than multi-objective. Operational techniques of decision analysis, such as multiattribute utility analysis, are specifically designed to deal with multiobjective problems like OY. I propose that a simple, linear, utility model be used to assess the optimality of alternative yield strategies in fisheries management. I illustrate the application of the model by assessing OY options in the New England herring (*Clupea harengus*) fishery and the Skeena River salmon (*Oncorhynchus* spp.) fishery. The advantages of the model are that it is simple and intuitively appealing, that it permits a wide range of types and qualities of data to be incorporated into the evaluation of management options, that it is amenable to sensitivity analysis, and that it is adaptable to a variety of decision rules.

L'auteur examine l'origine et la définition opérationnelle du concept de rendement optimal (RO) et décrit comment les techniques de l'analyse décisionnelle peuvent générer un modèle analytique du RO. En 1976, ce concept a été reconnu comme principe directeur de la gestion halieutique aux États-Unis et au Canada. Les politiques des deux pays précisent qu'une grande diversité de facteurs biologiques, économiques et sociaux doivent entrer en ligne de compte dans la détermination du RO. Il existe toutefois une certaine confusion quant au choix et à l'importance relative des facteurs qui devraient déterminer le RO de chaque pêche. Une certaine incertitude est aussi présente quant à la manière d'utiliser conjointement les facteurs économiques, biologiques et sociaux, comme l'exige le concept de RO. Les modèles biologiques et économiques établis ne sont pas adéquats pour une telle analyse car ils ne visent qu'un seul et non plusieurs objectifs. Les techniques opérationnelles de l'analyse décisionnelle, comme l'analyse utilitaire à attributs multiples, sont conçues précisément pour l'étude des problèmes à objectifs multiples comme le RO. L'auteur propose l'utilisation d'un modèle linéaire utilitaire simple pour évaluer l'optimalité d'autres stratégies de rendement dans la gestion halieutique. Il applique le modèle à l'évaluation de divers RO de la pêche du hareng (*Clupea harengus*) en Nouvelle-Angleterre et de la pêche du saumon (*Oncorhynchus* spp.) dans la rivière Skeena. Ce modèle a l'avantage d'être simple et intuitivement attrayant, de permettre l'incorporation d'une grande variété de types et de qualités de données lors de l'évaluation des choix gestionnels, de se prêter à l'analyse de sensibilité et d'être adaptable à une variété de règles décisionnelles.

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The management of fisheries is a complex task, involving the overt manipulation of an industry to achieve public goals. In the United States and Canada the stocks of marine and freshwater fishes have always been regarded as public property and it has been the responsibility of governments to administer their use. Until recently, access to the resource for private or commercial purposes was virtually unrestricted. Management measures to restrict fishing effort, when they were enacted, were usually justified on the grounds that depletion of the fish stocks must be prevented. Social goals, such as maximizing employment in the fishing industry or maintaining the economic viability of isolated coastal communities, were ofrten subsumed in management regimes. It was generally accepted, however, that the job of the fishery

manager was to ensure that a productive stock size was maintained. If this could be done with minimum interference to the industry it was assumed that the other things would work out for the best. The guiding principle of management was a biological goal, to maximize the sustainable yield (Larkin 1977). A rather elegant theoretical and operational technology developed in fisheries science to permit estimation of fish abundance and stock size for maximum yield.

During the last decade the policy of maximizing the yield of fish has given way to a policy of maximizing the net benefits to society from fisheries. Adoption of this policy has catapulted management agencies into the overt consideration of economic and social as well as biological factors in designing management regimes. While to a large extent it merely legitimized long standing concerns of fishery managers, the new policy has also created confusion and uncertainty. There is confusion over what nonbiological factors should be taken into account in designing management regimes, and who should decide what those factors are. And there is uncertainty over how to incorporate the multiple and conflicting goals implicit in the new policy into an objective evaluation of alternate management regimes.

The purpose of this paper is twofold. First, it is to sketch the evolution of fisheries management policy from emphasis on management of fish stocks to emphasis on management of fisheries. This evolution is exemplified by the change in the guiding principle of fisheries management from maximizing yield to optimizing yield. Much of the confusion and uncertainty that attends this new policy stems from the absence of an operational technology in fisheries to deal with the multiobjective nature of the optimum yield (OY) problem. My second objective, therefore, is to describe a technology that will permit any number of biological, economic, and social factors to be included in an objective evaluation of fishery management options. This technology is ideally suited to the problem of deciding what yield is optimum. I shall illustrate the technology with a discussion of OY in the New England herring (*Clupea harengus*) fishery and the Skeena River salmon (*Oncorhynchus spp.*) fishery.

The Evolution of Fisheries Policy

The scientific management of fisheries was born during the first quarter of this century (Baranov 1918) and grew during the second quarter with the developing theories of population ecology and population dynamics (e.g. Elton 1927; Russell 1931; Nicholson 1933; Ricker 1940; Delury 1947; Fry 1949). The guiding principle of fisheries management came to be the concept of maximum sustainable yield (MSY). For two decades following the second world war, research on methods for measuring population parameters and estimating maximum yield flourished. Classic papers like those of Ricker (1954) and Beverton and Holt (1957) highlighted this period. It culminated in handbooks for the calculation of biological statistics of fish populations that are familiar to every student of fisheries (Ricker 1958; Gulland 1969).

Implicit in the fisheries management literature predicted on MSY were three assumptions: (1) the dynamics of commercial fish stocks are sufficiently predictable that MSY is an achievable goal, (2) knowledge of fish stock dynamics is sufficient knowledge for effective resource management, and (3) MSY is an appropriate societal goal.

An occasional heretic questioned the singlemindedness of the MSY dogma and the missionary zeal (Larkin 1977) with which fishery managers pursued MSY. Challenges to the dogma did not carry much weight, however, until toward the middle of the third quarter of this century. By this time fishing technology had advanced to the point that fish stocks were being overexploited world wide. By 1975 it had become abundantly clear that, in most instances, stock dynamics were neither well enough understood nor sufficiently deterministic to render MSY an achievable goal, that knowledge of stock dynamics alone was not sufficient for effective management, and that MSY was probably not an appropriate societal goal anyway. Thus, the premises that made MSY a logical guiding principle of fisheries management had proven to be false, and the way was opened for the emergence of a new guiding principle.

In the United States and Canada, MSY was formally set aside as the guiding principle of fisheries management in 1976 with the publication in Canada of *Policy for Canada's Commercial Fisheries* (Department of Environment, Fisheries and Marine Service 1976) and passage in the United States of the Magnuson Fisheries Conservation and Management Act (MFCMA). Both of these documents state that the overall goal of fisheries management is to maximize the net benefits to society that can be derived from the fishery. The Canadian document is extremely prolix in describing what this means. Larkin's (1977) summary is as precise as any:

> ...The goals are to maximize food production, preserve ecological balance, allocate access optimally, provide for economic viability and growth, optimize distribution and minimize instability in returns, ensure prior recognition of economic social impacts of technological change, minimize dependence on paternalistic industry and government and protect national security and sovreignity...

The MFCMA is more concise. According to this legislation the goal of fisheries management is to achieve OY, which is defined as the amount of fish "(A) which will provide the greatest overall benefit to the Nation, with particular reference to food production and recreational opportunities; and (B) which is prescribed as such on the basis of the maximum sustainable yield from such fishery, as modified by any relevant economic, social, or ecological factor."

Clearly, the concept of MSY survives in these new policies, explicitly in the MFCMA and implicitly in the Canadian goal to maximize food production. But MSY is only one of several goals articulated in these documents. The new buzzword is "optimum yield" and it implies an amalgum of biological, economic, and social goals among which MSY could have a rather low priority.

Operationally Defining OY

The emergence of OY as a guiding principle of fisheries management constitutes explicit recognition in the United States and Canada of the multiobjective nature of fisheries management problems. The transition from MSY to OY in the operational sense, however, is proving difficult. The debate about what OY means and how it can be calculated has been extensive (Roedel 1975; Orbach 1977; Larkin 1977). The operational techniques that were developed to satisfy the MSY concept clearly could not be generalized to satisfy the OY concept. A recurrent complaint about the MFCMA, which legally constrains the Regional Fisheries Management Councils to harvest at OY, has been that the act is too vague. Alverson (1977) and Apollonio (1982) both noted that the act fails to state which social, economic, and ecological factors are to be taken

into account in determining OY, and what is the relative importance of these factors. Larkin (1977) voiced similar complaints about the lack of specificity in the Canadian policy document and in published definitions of OY. He warned that vagueness in the definition would inevitably lead to political manipulation of management. As we shall see later, however, the vagueness of the definition need not be a problem.

The MFCMA confuses the issue further by stating that OY in excess of domestic fishery needs must be allocated to foreign fleets. This seems to equate OY with surplus production, which was the basis of the MSY concept. The necessity of allocating "unused" OY to foreign fisheries was clarified somewhat by the Subcommittee on Fisheries and Wildlife Conservation and the Environment (Breaux 1981), which stated that this was not an absolute requirement of the legislation. The impression remains, however, that OY is a number of fish, determined in an unspecified way, but determined independently of decisions about how the fish are to be allocated. The concept of OY, however, implies that decisions about allocation are fundamental to determining what yield is optimum.

The operational definition of OY is equally confused in Canada. Recent Canadian policy documents (Department of Fisheries and Oceans, Fisheries and Marine Service 1981, 1982) continue to stress the importance of economic and social considerations in the fisheries. The operational technique, however, appears to be to calculate a total allowable catch (TAC) based on biological models of yield per recruit at $F_{0.1}$ (The rate of fishing mortality at which the increase in yield per recruit with a small increase in fishing mortality is $0.1 \times$ the rate of increase in yield per recruit at very low rates of fishing mortality, after Gulland and Boerema 1973). This TAC is then distributed among competing user groups in accordance with a set of guidelines that emphasizes such things as minimizing social and economic disruptions, satisfying the needs of native Indian fishermen, increasing economic viability and stability, encouraging independent ownership of fishing vessels, etc. Optimum yield in Canada, therefore, appears to mean a biological yield close to MSY distributed according to some set of social and economic guidelines. There is little discussion in Canadian policy documents about how regional management regimes could be tailored to meet these goals. Neither the MFCMA nor the Canadian policy documents give any guidance about how conflicting goals are to be reconciled or on what basis trade-offs are to be made.

The most incisive comments on OY occur in the literature on fisheries economics. Economists were among the first to challenge the emphasis on MSY in fisheries management (Taylor 1951) and among the first to employ the phrase optimum yield (Gordon 1953, 1954). When MSY was supplanted by OY the economists were ready with a theory and an analytic methodology for optimizing yield. The only viable approach, according to some economists (e.g. Turvey 1964; Crutchfield 1975; Pontecorvo 1977) was to equate OY with maximum economic yield (MEY). Four basic arguments have been used to support this point of view: (1) the real objective of fisheries is to benefit mankind, and economic yield is a recognized measure of benefits to mankind; (2) MEY always involves less effort in the fishery than MSY, so that stock conservation goals are generally met by managing to MEY, at least in single species fisheries; (3) any local social disruptions (e.g. redundancy in segments of the industry) consequent on rigorous management to MEY are small on a national scale, or even on a regional scale, and are insignificant compared with the theoretical economic benefits of not wasting labor and capital in the fishery; (4) although certain intangible nonmarket benefits of fishing may be ignored by the MEY paradigm there is, currently, no better way to capture the net benefits of fishing to society. Thus, managing to MEY, although not perfect, is the best we can do at the moment.

Despite these compelling arguments, MEY has not been adopted as the operational definition of OY. In fact, the MFCMA specifically guards against such a definition (National Standard No. 5: "Conservation and management measures shall, where practicable, promote efficiency in the utilization of fishery resources: except that no such measure shall have economic allocation as its sole purpose."). Nevertheless, economic analysis has become an important aspect of fisheries assessment and has had significant impact on the design of management regimes in recent years (Popper 1978; Needler 1979).

The impact of social factors on fishery management planning under the new policy is more difficult to assess. The importance of social factors beyond those captured by MEY is readily acknowledged (Crutchfield 1975, 1979; Christy 1977) and vigorously advocated (Orbach 1977, 1978; Pollnac and Littlefield 1983). However, no rigorous theory or set of empirical relationships has been put forward that would permit management regimes to be adjusted objectively and quantitatively for social considerations. A development along these lines with considerable potential is Pollnac and Littlefield's (1983) model of job satisfaction. While the empirical relationships presented by these authors are promising, they remain to be generalized into a prescriptive model of benefits from the fishery. The analytic tools available to the fishery manager remain, therefore, those for calculating MSY and MEY. In the current climate of disenchantment with MSY, it is not surprising that MEY has had a strong impact on recent management regimes for Canadian and United States fisheries.

My major objection to MEY as the operational definition of OY is that it amounts to substituting another single objective paradigm (maximizing return on capital and labor) for the old MSY paradigm. Adopting MEY as the operational definition of OY would mean sacrificing the great flexibility of the OY concept, flexibility which permits the weight given to biological, economic, and social factors in a fishery to be varied according to regional circumstances. The current approaches to determining OY in the United States and Canada do not capture this flexibility either. While they are not wholly based on either MSY or MEY, neither do they embody in a conjoint way the biological, economic, and social goals implicit in OY. It is the flexibility inherent in the OY concept that, if it could be captured within the bounds of a logical and objective analytical model, would constitute the greatest strength of OY as the guiding principle of fisheries management.

Thus, while the OY concept has considerable intellectual appeal, its practical application has been mired in controversy. The controversy revolves around the fact that no acceptable analytic methodology has emerged in fisheries management for ranking, weighting, and combining the multiple objectives that should determine OY. This has made the concept appear woolly, elusive, and, therefore, vulnerable to abuse (Larkin 1977).

Multiattribute Analytic Techniques

The failure of a multiattribute analytic methodology for dealing with problems like OY to emerge in fisheries is

surprising, since a well-developed methodology exists (Keeney and Raiffa 1976). This methodology has been effectively employed in other areas of natural resource management to resolve issues of resource allocation and to incorporate social, economic, and ecological goals into a single analysis (e.g. David and Duckstein 1976; Freeling and Seaver 1980; Ulvila and Seaver 1982). In a fisheries context, Bishop et al. (1981) argued for the use of multiattribute analytic techniques in designing fishery management regimes under the MFCMA, and Keeney (1977) and Hilborn and Walters (1977) applied one technique (multiattribute utility analysis) to the problem of managing Skeena River salmon. These papers appear not to have aroused much interest among fishery scientists or managers. Yet the analytic techniques proposed by these authors derive from a logically appealing and objective decision model that is useful for exploring a wide range of fishery management problems. The literature on multiobjective decision making is extensive. Here I shall attempt only to introduce the reader to the rich literature on multiattribute analytic techniques and show, through a discussion on OY in the New England Herring Fishery and the Skeena River salmon fishery, how these can provide an analytic framework for defining OY.

The New England herring fishery and the Skeena River salmon fishery are quite different in character. The New England sea herring fishery currently harvests mainly from one stock of herring (the Gulf of Maine stock) although two other stocks (the Georges Bank and Southwest Nova Scotia stocks) contribute to the catch. The fishery is particularly important to Maine and Massachusetts but New Hampshire and Rhode Island also have a stake in the fishery. The bulk of the Maine fishery is an inshore fishery using fixed gear to catch juvenile herring for the sardine trade. This fishery is particularly important in the economy of isolated coastal communities in Maine. The fisheries in the other states are mobile gear fisheries for adult herring to supply filleting and specialty product markets. Adult herring are caught partly in state territorial waters where they are under the jurisdiction of the state management agencies and partly in the Fishery Conservation Zone where they are under the jurisdiction of the New England Fishery Management Council (NEFMC). The stocks supporting the fishery migrate back and forth across the international boundary and are harvested by both Canadian and U.S. fishermen. Thus, the herring fishery is an example of a single-species fishery involving multiple stocks, multiple user groups with conflicting objectives, and several political jurisdictions.

Keeney (1977) described the Skeena River fishery. Briefly, it involves five species of Pacific salmon and one species of anadromous trout (steelhead, *Salmo gairdneri*), a commercial fishery prosecuted by three different gear types, an active sports fishery, and a native Indian subsistence fishery. The Department of Fisheries and Oceans (DFO) is the single regulatory agency responsible for management of the fishery. The fishery is economically important to the Skeena region. Thus, the salmon fishery is a multispecies fishery involving several user groups but only one regulatory agency. Both the herring and the salmon fisheries present complex, multiobjective management problems involving important biological, economic, and social concerns.

The analytic models used in designing and assessing management regimes for both these fisheries have been of the traditional single objective type. Yet managers have clearly had more than one goal in mind in the management of these fisheries. Presumably, a multiattribute analytic solution would have been useful to them.

The general analytic solution to the multiobjective problem may be stated simply as follows:

$$(1)\quad U_i = \left[\sum_j a_j^p S_{ij}^p\right]^{1/p}$$

where U_i is an aggregate measure of the performance of the ith solution (e.g. management regime) against $j = 1 \ldots n$ attributes of the problem, a_j is the preference weight associated with the jth attribute of the problem, S_{ij} is a measure of the performance of the ith solution against the jth attribute, and p is an integer specified in the range $1 - \infty$ (usually 1, 2, or ∞) depending on the choice of decision rule and the choice of performance measure for each solution. In this formulation, p can only take values other than 1 if S_{ij} is a measure of the difference between the actual score of a policy i on an attribute j and the ideal score for attribute j.

Here I shall discuss only solutions in which

$$p = 1,\ \sum_j a_j = 1,$$

and S_{ij} scores are scaled from 0 (worst) to 100 (best), i.e.

$$(2)\quad U_i = \sum_j a_j S_{ij}.$$

This is the linear weighted solution to the optimization problem in which the optimum solution is the solution that maximizes U. Cohen and Marks (1975), Keeney and Raiffa (1976), Starr and Zeleny (1977), Bell et al. (1977), and Duckstein and Opricovic (1980) discussed the application and interpretation of numerous other optimization solutions.

This analytic framework finds a natural application in the problem of defining OY. The concept of OY implies that biological, economic, and social factors are all to be taken jointly into account in determining the yield to be taken from the fishery. We can specify the factors that are important to us, those that should play an important role in determining OY. These become the attributes, or criteria, that we will use to evaluate the overall suitability of any particular yield of fish. For any specified set of attributes we can, either objectively or subjectively, determine how well any particular yield of fish satisfies each attribute (the S_{ij} of eq. 2). We may also presume that not all attributes are equally important to us and that we can specify our preference weightings, or that we can research the preference weightings of any affected group, for the set of attributes (the a_j of eq. 2). The optimum yield is that yield that maximizes U in eq. 2.

This process constitutes a practical application of multiattribute utility theory (MAUT). There are essentially six steps in the application of this theory to the resolution of multiobjective problems like determining OY (Table 1).

Bounding the Problem

The first step is that of bounding the problem, deciding what is to be included in the analysis and what is to be left out. In the case of OY, there are three primary dimensions along which to bound the problem, biological, economic, and social. There are no hard and fast rules for establishing boundaries; it is wholly a matter of judgement on the part of the fishery manager. The choice of problem boundaries should not be regarded as a trivial

TABLE 1. The six steps in the application of multiattribute utility theory to determining optimum yield.

1. *Bounding the problem:* Deciding which factors and which constituencies are to be taken into account and which are to be left out in determining optimum yield
2. *Determining the feasible policy alternatives:* Deciding what is the range of technically feasible yields within which the optimum yield must lie
3. *Deciding on the attributes of the problem:* Selecting a comprehensive, mutually exclusive, and preferentially independent set of attributes against which the feasible yields will be judged
4. *Setting the attribute weights:* Determining the relative importance of the attributes as criteria for distinguishing among yields
5. *Scoring the policies:* Objectively scoring each feasible yield against each attribute
6. *Applying the decision rule:* Combining the scores and attribute weights for each yield according to the predetermined decision rule and selecting the optimum yield

matter, however. The decision about what will be included and what left out of the analysis is likely to affect the optimum yield, and a poor choice of boundaries could come back to haunt the manager when he tries to implement his OY policy. On the other hand, since the analysis is quantitative, the manager can evaluate a variety of boundary decisions and search for unexpected consequences (Holling 1978).

A significant aspect of bounding the OY problem is to decide who has a stake in the management regime, and who are the constituencies the regime is expected to serve. For example, Keeney (1977), in his MAUT analysis of the Skeena River salmon fishery, identified five principal affected groups, four "types" of fishermen and a composite regional group whose welfare was tied to fishing such as cannery operators, lodge operators, etc. (Table 2). The definition of these groups was restricted to the Skeena region. Clearly, other groups and geographic boundaries could have been devised. Hilborn and Walters (1977) reviewed a broader group of constituencies in their analysis of enhancement options for the Skeena River. I have also listed some groups outside the Skeena region who have an important stake in the fishery (Table 2). Keeney's way of bounding the problem reflected what he saw as the primacy of the well being of regional fishermen to regional management decisions, the secondary importance of other regional activities associated with fishing, and the relative unimportance of affected groups outside the region. The aggregation of lodge owners, cannery operators, etc., into a regional development group also reflected a judgement by Keeney that the members of this group would react similarly to any potential policies.

In a similar way, I have identified groups likely to be affected by management of the sea herring fishery in New England (Table 2). Like Keeney (1977), I have kept the fishermen segregated by gear type. Herring fishermen could be aggregated into offshore and onshore groups, since these groupings also reflect state and product dichotomies. A herring management plan prepared in 1977 (NEFMC 1978), however, showed that management regimes can affect the gear types differently. It seemed, therefore, appropriate to disaggregate the onshore and offshore fisheries. I have also disaggregated the processors into sardine, fillet, and meal processors, since sardine processing

TABLE 2. Groups considered to have a stake in decisions about optimum yield in the Skeena River salmon fishery and the New England herring fishery.

Skeena salmon fishery	New England herring fishery
Fishermen	
Lure fishermen (trollers)[a]	Offshore fishermen (seiners, pair trawlers)[b]
Net fishermen (seiners, gillnetters)[a]	Onshore fishermen (wiers, stop seiners, purse seiners[b]
Sport fishermen[a]	
Native Indians	
Other regional groups whose welfare is tied to fishing	
Cannery companies[a]	Sardine canneries[b]
Motel operators[a]	Fillet processors[b]
Sport lodges, etc.[a]	Fish meal processors
	State legislators
	State fishery managers
Groups outside the region whose welfare is tied to fishing	
Federal legislators	Foreign fishermen
Provincial legislators	Federal legislators
Public at large	Public at large
Fishermen and processors in other regions	National Marine Fisheries Service
British Columbia Hydro Authority	

[a]Groups considered by Keeney (1977) in his MAUT analysis.
[b]Groups considered by the NEFMC in developing their 1978 herring management plan.

occurs predominantly in Maine and fillet and meal processing predominantly in Massachusetts. I segregated meal processors because state statutes require that food uses of herring take precedence over reduction. Other regional groups with a stake in herring management include state legislators and state fishery management agencies. Groups outside the region include foreign fishermen, federal legislators, National Marine Fisheries Service (NMFS), and the public at large. The inclusion of the regulatory agencies, or even the decision making body itself (e.g. DFO in Canada or the NMFS and the Regional Councils in the United States) among the affected groups is, in my view, an important part of the accounting process. The prestige accorded these groups and the self-esteem of their members is affected in important ways by the mangement regimes that are adopted. It would be naive to imagine that the regulatory agencies are indifference to the management regime and will not use their position to influence the regime.

The set of affected groups that I have identified for the problem of OY in herring and salmon is rather broadly defined. This is to emphasize the range of choices open to the manager and the need for careful consideration in identifying whose concerns are to be taken into account in designing the management regime and what are their natural groupings. For example, the groupings in Table 2 emphasize technological or functional associations. In other instances, geographic or community associations might be more appropriate. By whatever means the final list is determined it should include all those groups whose views are likely to influence the final decisions about fishery management. The importance of carefully selecting these "stakeholder" groups will be more important later on. Suffice it to say at this stage that their preference structures will have an important impact on the determination of OY.

A second important aspect of bounding the OY problem is to decide on the time horizon for OY. Since fish are a common property resource, management agencies typically have a strong conservation mandate, and their tendency is to adopt a long time horizon. Despite the fact that, for the sake of expediency, sustainable yield was often exceeded, the goal of management in the past was always to achieve a yield close to MSY. Even though MSY has now been replaced by OY, both U.S. and Canadian policy still asserts that any optimum yield must be a yield that can be taken on a "continuing" basis. What continuing means, however, is left to the discretion of the fishery manager and this opens the door to management options that include short-term overfishing. A variety of circumstances can be imagined in which the short-term advantages of overfishing outweight the benefits of a longer term view. Provided full account is taken of the price that must be paid later if sustainable yield is exceeded now, I see no objection to serious consideration of such options. In fact, the multiattribute analytic techniques that I am discussing are ideally suited to evaluating the trade-off between short- and long-term benefits of a management option in a realistic way. For the purposes of this paper, however, it is appropriate and simpler for me to take a long-term view, as this will permit me to use traditional equilibrium fisheries models in my discussion. This is not to discount the importance, or the difficulty, of short-term decision making. Often the most contentious decisions the manager will have to make will involve what to forego now to achieve increased future returns. Suffice it to say that this trade-off could also be incorporated into the analysis but at the cost of a considerable increase in complexity.

Other aspects of bounding the problem include the more familiar fishery management tasks of deciding geographic and stock boundaries and delineating important ecosystem interactions that might be influenced by a change in management regime. For the New England herring fishery the stock boundaries are the three broadly defined stocks of herring found in the Gulf of Maine: The Southwest Nova Scotia stock, the Georges Bank stock, and the Gulf of Maine stock. Geographic boundaries extend from Rhode Island to Maine and from the Shoreline to 200 mi (320 km) offshore. These are generally the boundaries set by the NEFMC in their 1978 fishery management plan for herring.

For the Skeena River salmon fishery the stock boundaries include the six species of Pacific salmon and anadromous trout that spawn in the river and its tributaries. The geographic boundaries include the watershed of the Skeena River and the marine waters off northern British Columbia which comprise the nursery and migratory pathways for the salmon.

Determining the Range of Feasible Yields

The second step in MAUT is to determine the set of feasible policy alternatives (Table 1). In the case of OY this means deciding on a set of feasible yields. Here I shall assume that the Schaefer model (Ricker 1975) is an adequate description of the long-term relationship between fishing effort and yield for both the herring and salmon fisheries, and that all yields from 0 to MSY are technically feasible. Thus, the feasible set of yields, which includes OY, ranges from 0 to MSY. It is important to remember, however, that for every yield other than MSY there are two possible levels of effort, and thus, two possible sets of economic, social, and ecological values.

The determination of feasible yields for the herring fishery is complicated by the fact that not only are there three stocks contributing to the fishery but also that the juvenile and adult fisheries harvest different components of the same stocks. Fortunately, it appears that maximum yield per recruit occurs at about the same fishing mortality for all the stocks. Strictly on the basis of yield per recruit, the adult fishery has the potential to provide the greatest biomass of harvest (Fogarty et al. 1981). The juvenile fishery is an old and established component of the fishery, however, and is economically important to the State of Maine. Part of the problem of OY then is to determine the appropriate trade-off between the adult and juvenile fisheries.

While all yields between 0 and MSY are feasible, and all combinations of juvenile and adult fisheries, I shall restrict my analysis to three points on the yield curve, MEY, MSY, and the bionomic equilibrium. I shall also restrict my analysis to three combinations of adult and juvenile harvest, all juvenile, all adult, and 50:50 juvenile and adult. This gives a total of nine different yield policies for comparison. While none of these may be strictly optimum, they will suffice to illustrate the method. In addition, their relationship to one another may point the way to a true optimum.

Selecting an illustrative set of feasible yields for the Skeena River salmon fishery is more difficult. All six salmon species are highly vulnerable to the suite of modern gear in the fishery so that yields from 0 to MSY are certainly feasible. The species differ in productive capacity, however, ranging from less than two recruits per spawner for chum (*O. keta*) to approximately four recruits per spawner for chinook (*O. tshawytscha*), they differ in their vulnerability to the various commercial and recreational fishing gear, and they differ in their market value. The determination of OY in the salmon fishery is, therefore, much more dependent on how the fish are allocated than is the determination of OY for herring. This makes it difficult to choose a small set of yields for the six species that captures the essence of the OY problem in this fishery.

Some simplifying assumptions must be made. All the species in the Skeena River fishery are presently overfished, lightly in the case of sockeye (*O. nerka*) and pink (*O. gorbuscha*) salmon, the most abundant species, moderately in the case of coho (*O. kisutch*), and heavily in the case of chum, chinook, and steelhead. The majority of the catch is taken in the commercial fishery. I shall assume, therefore, that the most critical trade-offs affecting OY will be the allocation trade-offs among the commercial gear types, and the trade-off between maintaining current levels of commercial fishing effort with catches below MSY or reducing effort to obtain greater catches in the long run. This is not to say that the allocation trade-off between commercial/recreational/subsistance fisheries should not be a concern of the manager. It only means that I shall ignore this trade-off in my sample analysis. A thorough analysis of OY in the salmon fishery would certainly take this trade-off into account just as it would take account of the trade-off between short- and long-term costs and benefits as noted earlier. I shall take the point of view that the recreational and subsistance fisheries will both benefit from a restriction in commercial effort and an increase in salmon runs to the Skeena.

The illustrative yields for the Skeena River salmon fishery will be, therefore, the current biomatic equilibrium yields for all species (estimated as the recent average yields in the fishery) and the yields that can be projected to result from about a 30% decrease in total commercial fishing effort (close to the combined species MSY). I shall combine these yields with three

TABLE 3. Attributes of optimum yield for the Skeena River salmon fishery and the New England herring fishery and the range over which each attribute might vary with different management policies.

Skeena salmon			New England herring		
Attribute	Best	Worst	Attribute	Best	Worst
			Biological and conservation goals		
Spawning escapement (thousands)			Stock size (MT × 10^3)		
Sockeye	900	300	Southwest Nova Scotia	500	200
Pink	1500	500	Georges Bank	500	200
Chum	50	5	Gulf of Maine	110	40
Coho	150	20	Predator stock (relative)	2.0	1.0
Chinook	60	10	Fish species diversity	1.9	1.5
Steelhead	50	5			
			Economic goals		
Net fishermen income ($ × 10^3)	50	0	Offshore fishermen income ($ × 10^3)	90	0
Lure fishermen income ($ × 10^3)	50	0	Inshore fishermen income ($ × 10^3)	12	0
Recreation income ($ × 10^6)	10	0	% profit sardine processor	10	2.0
Cost of salmon ($/lb.)	0.2	10	% profit fillet processor	15	7.0
Management cost ($ × 10^6)	0	10	Management cost ($ × 10^6)	0	2.5
			Social Goals		
Days fishing (nets)	100	0	Harvest sector jobs	500	150
Days fishing (lures)	100	0	Processing sector jobs	1500	500
Sport catch (×10^6)	1.0	0	Days fishing	150	30
Indian catch (×10^3)	200	0	Days processing employment	200	60
Regional jobs	5000	0	Trip length (days)	1.0	3.0
			Plant environment (relative)	2.0	1.0
			Injuries (annual)	0	20
			Foreign catch (MT × 10^3)	60	0

allocation options for the commercial fisheries: (1) allocation according to the recent landings by each gear type, (2) allocation of more sockeye and pink to the lure fishery in return for a reduction in effort by the lure fishery on chinook, coho, and steelhead, and (3) allocation of the total catch to the net fishery. This gives a total of six different yield alternatives. While this is a small set of the potential alternatives for this fishery, it is a set that touches on some of the important management concerns in the Skeena River fishery and should give a sense of the power of MAUT in evaluating policy alternatives for such a complex multispecies fishery.

Establishing the Attributes of the Problem

The third step in MAUT (Table 1) is to establish the attributes of the problem. These are the criteria against which the yields will be judged in order to determine which is optimum. Just as decisions about inclusion or exclusion of stakeholders were judgemental, so are decisions about which attributes adequately describe the problem. There is no unique set of attributes for any OY determination. The set that is chosen, however, must meet four criteria: (1) it must be comprehensive; i.e. it must reflect all aspects of the problem as it is bounded; (2) the attributes must be mutually exclusive, i.e. the same things may not be counted twice; (3) the attributes must be independent, i.e. the preference for a particular trade-off between any two attributes should not be dependent on the amount of any other attribute present in the system; and (4) the attributes must be additive, a scaling problem for which there are a number of solutions.

Keeney (1977) defined 12 attributes of the Skeena River salmon management problem. He arrived at these by taking advantage of the hierarchical nature of goals. As I noted earlier, Keeney focused on regional needs in his analysis, and the attributes he chose reflect this. The only attribute in Keeney's (1977) set not designed to satisfy some regional economic or social goal was the annual government expenditures on management. DFO, which administers the Skeena fishery, has, however, a responsibility for resource conservation that goes beyond satisfying the immediate needs of the resource industry. For example, a policy that clearly promoted the extinction of one or more of the species of salmon in the Skeena River would be unacceptable even if the policy scored very high on the set of regional attributes Keeney identified. I have, therefore, added an attribute for the spawning escapement of each species to Keeney's list and dropped the attribute "number of salmon species." The total number of attributes for the Skeena OY problem is then 16 (Table 3). There is also a range of possible values that the attributes might take depending on the management policy (Table 3). I have set the lower bounds on escapement for each species above 0 to reflect the probable lower limit of escapement that would precipitate drastic management action by DFO. Keeney (1977) discussed the range of possible values for the other attributes and their relevance to the problem of managing the Skeena River fishery.

The hierarchical nature of goals also provides a way to decompose the OY problem for New England herring into a set of attributes (Table 4). Twenty attributes appear to describe the herring OY problem (Table 3). Five of these are biological. A major concern of herring managers is the possibility of stock collapse. There are no agreed upon or infallible indicators of stock collapse, but the probability of collapse is presumably inversely related to stock biomass, so I have included stock biomass as a proxy indicator of probability of collapse. Genetic diversity is a long-standing concern of fishery managers, which

TABLE 4. A hierarchy of goals for the New England herring fishery.

Maximize net benefits to society		
Conservation of resources goals	Economic development goals	Social development goals
1. *Conserve target species:* Maintain productive stock biomass for Georges Bank, Gulf of Main, and Southwest Nova Scotia stocks	1. *Maximize net income:* Offshore fishermen, onshore fishermen, sardine processors, fillet processors, meal processors	1. *Maximize employment in the industry:* harvesting jobs, processing jobs
2. *Conserve other valued species:* maintain biomass of commercially valuable herring predators	2. *Minimize costs:* Enforcement costs	2. *Maximize satisfaction:* Days fishing, days processing employment, trip length, plant environment
3. *Conserve marine ecosystem:* maintain species diversity of Maine/Georges Bank	3. *Maximize economic efficiency:* Economic rent	3. *Maximize safety:* Injuries to fishermen and processors
		4. *Foreign relations:* Foreign catch

has become even more fashionable lately (Berst and Simon 1981), and is now measurable by a number of techniques. The concern for genetic diversity is satisfied by disaggregating the three stocks of herring and considering their biomasses separately. Concern for interaction among species of commercial value and the possibility that reduced herring biomass might affect the forage base for predatory commercial species, like the cods, is satisfied by the inclusion of predator biomass as an attribute. Concern for the effects of man's harvesting activities on the ecosystem in general is satisfied by the inclusion of fish species diversity (Shannon–Wiener Index) as a proxy measure of ecocystem stability (Tables 3, 4).

There are seven economic attributes. These include net income to fishermen and processors disaggregated by gear and product type to satisfy concern for how the total income from the fishery is distributed. The concern of regulatory agencies over the costs of administering any regime is satisfied by the inclusion of enforcement costs as an attribute. The concern of economists over the efficient use of capital and labor in the fishery is satisfied by inclusion of economic rent as an attribute. Regional legislators are likely to be concerned about continued economic development in the fishery. In my view this concern is also satisfied by the attribute of economic rent, since presumably any rent generated by the fishery will either be reinvested in the fishery or in other regional developments.

There are eight social attributes. These include employment opportunities in both the harvesting and processing sectors. Harvesting and processing employment are kept separate because the harvesting sector employs predominantly men, while the processing sector employs predominantly women. The attributes of job satisfaction for fishermen are days spent fishing and length of individual trips, two factors that Pollnac and Littlefield (1983) found closely related to fishermen satisfaction. Similar attributes score for job satisfaction among plant workers. I have considered safety to be a relevant management goal with injuries to fishermen and processors as the attribute. The issue of foreign fishing poses some problems, as much of the impetus for the MFCMA was to eliminate foreign fishing. For some stakeholders any amount of foreign fishing is bad, while for the State Department the licensing of foreign fishing provides some international leverage. Also, the Law of the Sea convention prohibits coastal states from denying access to foreign fishermen if there are identifiable unutilized fishery resources in coastal waters. From a purely nationalistic point of view there is no reason ever to permit foreign fishing within territorial waters. Such fishing will only reduce the catch-per-unit-of-effort of national fishermen and provide competition in the marketplace for domesticly produced goods. Thus, in my view, foreign fishing is a social issue that falls in the context of foreign relations. The value of fish as a foreign relations tool must be considered in relation to their value if used some other way, even if that alternate use is simply to provide a cushion against the possibility of stock collapse.

Weighting the Attributes

The fourth step in MAUT (Table 1) is setting preference weights on the attributes, the a_j's of eq. 2. In a decision problem with as many powerful stakeholder groups as there are in fisheries, determining a set of preference weights acceptable to all groups is likely to be difficult. The first step is to assess the specific preference pattern of each stakeholder. Nominally, this is an iterative process whereby the stakeholder first ranks the attributes from the least to the most important. Then the stakeholder assigns successively to each attribute, from the least important to the most important, a score that reflects the relative importance of that attribute to the one next below it in rank. Giving the lowest ranking attribute a score of 10 provides some flexibility in scoring those attributes immediately above it (Edwards and Newman 1982). Careful cross referencing among the attributes and their scores is required to ensure that the final weights are a true reflection of the preferences of the stakeholder. It is also important in assigning weights to attributes that the stakeholder consider not only the philosophical importance of an attribute but also the likely impact of any feasible policy on values of the attribute. If none of the feasible

TABLE 5. Weights for the attributes of optimum yield in the Skeena salmon fishery and the New England herring fishery.

Skeena salmon fishery		New England herring fishery	
Attribute	Weight	Attribute	Weight
Spawning escapement		Conservation	
Sockeye	0.04	Southwest Nova Scotia stock	0.09
Pink	0.04	Georges Bank stock	0.12
Chum	0.08	Gulf of Maine stock	0.16
Coho	0.08	Predator stocks	0.03
Chinook	0.11	Species diversity	0.01
Steelhead	0.10	Subtotal	0.41
Subtotal	0.45		
Income and costs		Income and costs	
Net fishermen	0.08	Offshore fishermen	0.09
Lure fishermen	0.08	Inshore fishermen	0.09
Recreational revenue	0.07	Sardine processors	0.08
Cost of salmon	0.02	Fillet processors	0.05
Cost of management	0.05	Meal processors	0.02
Subtotal	0.30	Cost of management	0.03
		Economic rent	0.01
		Subtotal	0.37
Social satisfaction		Social satisfaction	
Days fishing nets	0.02	Harvesting jobs	0.05
Days fishing lures	0.02	Processing jobs	0.04
Sport catch	0.05	Days fishing	0.02
Indian catch	0.06	Trip length	0.02
Regional jobs	0.10	Days processing work	0.02
Subtotal	0.25	Plant environment	0.02
		Injuries	0.02
		Foreign catch	0.03
		Subtotal	0.22

policies is likely to have much impact on an attribute then it should receive a low rank and a low weight regardless of its philosophical importance. Species diversity is potentially such an attribute in the herring OY problem (Table 3). Important as the concept of ecosystem stability might be, there is little likelihood of any OY having a large impact on species diversity among the fishes of the Gulf of Maine and Georges Bank, even though herring is an important forage fish. The reverse is, of course, also true. Attributes of moderate philosophical importance should receive greater weight if their value is likely to be strongly influenced by the range of feasible policies.

Once the attributes are ranked and assigned their relative weights the weights may be normalized so that their sum equals 1.0. This is done simply by dividing each weight by the sum of weights for all attributes. This step, although not essential, keeps the aggregate utility scores in the range 0–100.

Differences in preference patterns among stakeholders are inevitable and these may be sufficient to affect significantly the choice of OY. According to Gardiner (1981), however, the MAUT process encourages agreement among the stakeholders when compared with wholly intuitive approaches to policy evaluation. This is because, in the absence of a structured decision analytic model, stakeholders tend to key on attributes that are most important to them and to discount heavily, or disregard, other attributes of the problem. Nevertheless, the weighting process may reveal substantial disagreement among stakeholders (e.g. Hilborn and Walters 1977). The operational decision maker (the Regional Councils in the United States and DFO in Canada) will have to take such disagreements into consideration when developing OY. Particularly important will be the degree to which such disagreements may frustrate attempts to achieve OY in the fishery. Affected groups may, for example, refuse to comply with regulations they regard as unfair.

One approach to resolving disagreement among stakeholder groups is to include those groups with conflicting preference structures as a level in the hierarchy of goals. A goal of the analysis then becomes to satisfy maximally the desires of the conflicting groups. This was the approach taken by Keeney (1977) in developing a utility function for the Skeena salmon problem. In this approach the central decision maker must determine a set of preference weights for the trade-off among conflicting stakeholders. Any arbitrary assigning of weights to the affected groups, however, may be regarded as unfair and could result in just as much opposition to the OY solution as would have been generated by the original preference conflict. A more satisfactory approach may be to confront the stakeholders with the differences in their preference weights and negotiate a compromise.

In the case of the Skeena fishery, I determined the preference weights for use in the sample OY analysis from preference data in Keeney (1977) and my knowledge of this fishery (Table 5). In the case of the New England herring fishery, I determined preference weights from informal discussions with NEFMC members and detailed discussions with Dr Susan Peterson (Woods Hole Oceanographic Institution, Woods Hole, MA), an anthropologist who served on the Scientific and Statistical committee of the NEFMC for several years.

For both fisheries the combined weight of conservation attributes is greater than the combined weight of either economic or social attributes alone (Table 5). This reflects the emphasis that most fishery managers place on resource conservation. The combined weight of social and economic attributes taken together is greater than that for conservation goals, however, indicating that policies that totally disregard economic and social attributes will never win out over those that emphasize these attributes at the expense of some conservation goals.

Scoring the Yields

The fifth step in MAUT analysis is to score the policy options against the attributes they must satisfy (Table 1). I derived scores for the biological and economic attributes from the familiar bionomic relationships based on the Schaefer model (Fig. 1).

The yield curves in Fig. 1 for the six salmon species in the Skeena River are an approximate representation of surplus production based on unpublished information in reports of geographic working groups within DFO. The relative heights of the curves are in proportion to projected maximum yields for each species in the Skeena. The total yield curve is simply the sum of the individual yield curves. The cost line was drawn from the origin to intersect the total yield curve at a level of effort consistent with current yields in the fishery and the degree of overexploitation of the individual species. The yield curves for chum, coho, chinook, and steelhead are scaled along the effort axis to reflect their vulnerability to the suite of gear in the fishery and their current levels of overexploitation, high for chum, chinook, and steelhead, moderate for coho.

The yields of each species at current fishing effort (E_2, Fig. 1) and fishing effort for MSY (E_1, Fig. 1) can be deduced from the figure (Table 6). Escapement may be presumed proportional to yield. Although not all the yield curves are symmetrical, escapement for all species appears closer to optimum, as defined by the geographic working groups, at E_1 then at E_2. I have presumed, however, that the allocation option that eliminated the lure fishery but maintained total effort at E_2 would potentially permit significant recovery of the coho, chinook, and steelhead stocks, since effort in the net fishery could be directed more toward pink and sockeye. This allocation option, therefore, scores well on escapement for the heavily exploited species.

Income for net and lure fishermen is presumed proportional to catch with some adjustment for different prices paid for the different species. The alternative that allocates a greater share of sockeye and pink to lure fishermen presumably would reduce the income of net fishermen, while the alternative that eliminates the lure fishery presumably drops their regional income to zero. Sport catch I presumed to be proportional to escapement of the principal recreational species (coho, chinook, and steelhead) and Indian catch proportional to total escapement. I presumed regional jobs to be approximately proportional to total catch but adjusted somewhat for elimination of the lure fishery in some policies (Table 6).

The yield curves for adult and juvenile herring were derived from Fogarty et al.'s (1981) yield per recruit analysis (Fig. 1). The relative positions of the cost lines are subjective and based on information in Hu et al. (1983) and the Sea Herring Fishery Management Plan (NEFMC 1978). The positions of the nine yield alternatives that I shall analyse (MEY, MSY, and

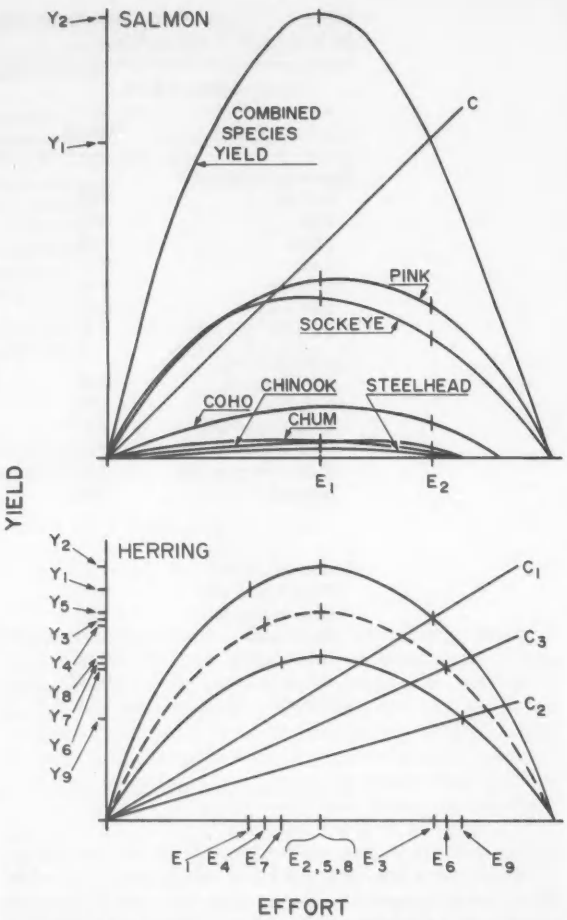

FIG. 1. Yield (fish or $) and cost ($) in relation to fishing effort for the Skeena River salmon fishery and the New England herring fishery. The cost line for the salmon fishery is drawn in relation to the combined yield line for all species. Effort level E_1 (yield Y_1) for the salmon fishery is the effort level for the combined species MSY, while E_2 (yield Y_2) is the current bionomic equilibrium. The relative yields by species at each of these effort levels may be determined from the individual species yield curves. The yield curves for the herring fishery represent an all adult fishery (upper curve), an all juvenile fishery (lower curve), and a 50:50 adult/juvenile fishery (broken curve). Cost lines C_1–C_3 represent harvesting costs for the adult only, juvenile only, and mixed fisheries, respectively. Effort levels E_1–E_9, and corresponding yields Y_1–Y_9, represent effort and yield for MEY, MSY, and bionomic equilibrium in the adult only, mixed, and juvenile only fisheries, respectively.

bionomic equilibrium for an all juvenile, all adult, and a 50:50 juvenile/adult fishery) are shown in Fig. 1. I deduced scores for the biological and economic attributes of the herring OY problem from Fig. 1 and information in Fogarty et al. (1981), Hu et al. (1983), and the Sea Herring Fishery Management Plan (NEFMC 1978) (Table 7). I also based scores for the social attributes on information in these documents about employment in the various sectors of the industry and working and fishing conditions in the industry.

It was a fairly simple matter in either fishery to identify the

TABLE 6. Scores of yield alternatives against attributes for six alternatives in the Skeena River fishery (scores are scaled form 0 (worst) to 100 (best)).

	Current level of effort: Allocation option			MSY level of effort: Allocation option		
	(1) Status quo	(2) More pink and sockeye to lures	(3) No lure fishery	(4) Status quo	(5) More pink and sockeye to lures	(6) No lure fishery
Biological attributes						
Sockeye	0	0	0	100	100	100
Pink	0	0	0	100	100	100
Chum	0	0	20	60	100	80
Coho	0	20	60	50	80	100
Chinook	0	20	60	50	60	100
Steelhead	0	20	60	50	60	100
Economic attributes						
Net fishermen ($)	40	0	60	80	60	100
Lure fishermen ($)	10	0	60	80	60	100
Recreational ($)	0	20	40	80	90	100
Cost of salmon	0	0	0	100	100	100
Management cost	40	0	80	60	20	100
Social attributes						
Days net fishing	20	0	60	80	60	100
Days lure fishing	70	80	0	90	100	0
Sport catch	0	20	60	50	80	100
Indian catch	0	20	40	60	80	100
Regional jobs	0	20	60	80	100	90

TABLE 7. Scores of yield alternatives against attributes for nine alternatives in the New England herring fishery (scores are scaled from 0 (worst) to 100 (best)). MEY, maximum economic yield; MSY, maximum sustained yield, BE, bionomic equilibrium.

	Adult fishery			50:50 Adult/Juvenile			Juvenile fishery		
	(1) MEY	(2) MSY	(3) BE	(4) MEY	(5) MSY	(6) BE	(7) MEY	(8) MSY	(9) BE
Biological attributes									
Southwest Nova Scotia stock	100	65	10	92	65	5	85	65	0
Georges Bank stock	100	65	10	92	65	5	85	65	0
Gulf of Maine stock	100	65	10	92	65	5	85	65	0
Predators	100	65	10	92	65	5	85	65	0
Species diversity	100	65	10	92	65	5	85	65	0
Economic attributes									
Offshore fishermen	100	75	50	50	37	25	0	0	0
Inshore fishermen	0	0	0	50	37	25	100	75	50
Sardine processors	0	0	0	47	50	30	95	100	60
Fillet processors	90	100	78	46	50	39	0	0	0
Meal processors	40	48	0	71	74	16	91	100	31
Management costs	75	37	0	100	62	25	87	50	12
Economic rent	100	86	0	83	76	0	69	67	0
Social attributes									
Harvesting jobs	0	12	25	37	50	75	62	87	100
Processing jobs	0	25	12	50	62	37	87	100	75
Days fishing	0	12	25	37	50	62	75	87	100
Trip length	100	75	0	100	75	0	100	75	0
Days processing	0	25	12	50	62	37	87	100	75
Plant environment	87	100	75	50	37	62	12	25	0
Injuries	87	100	25	62	75	0	37	50	12
Foreign catch	87	100	75	50	62	37	12	25	0

best and the worst performer among the yield alternatives for a particular attribute. I gave these yields scores of 100 and 0, respectively, on that attribute. It was also, in all instances, possible to rank order the other yields between these extremes. For the relationships depicted in Fig. 1, I could determine the relative value of a particular yield on an attribute and assign scores according to those values on the scale of 0–100. Where I could only determine rank ordering, I assigned scores at equal intervals along the scale 0–100.

Obviously, where quantitative production and economic models exist for a fishery these should be used in scoring yield alternatives. Subjective decisions may still have to be made about the impact of a particular alternative on some attributes. Additionally, there may be strong differences of opinion among experts about the interpretation of quantitative models and empirical data, so that several "objective" scores are available for a particular attribute. A thorough analysis should always test the sensitivity of the model to contentious scores. The scores that I have assigned are rather subjective and this could affect the outcome. The skeptical reader, however, may easily determine how a change in any score might influence the analysis.

Since the analytic model is not sensitive to either the units or the original scale of measurement, attributes for which there is no clear empirical evaluator may be included in the analysis. An example of such an attribute might be the value of the "recreational experience" associated with sport fishing or the "social value" of fishing as an occupation. Scoring alternative policies against such attributes may only be possible by soliciting expert opinion. Other attributes, for which there is a clear objective scale of measurement, but insufficient data to permit precise location of an alternative on the scale, may also have to be scored by expert opinion. Approaches to the use of expert opinion differ. Edwards (1971), for example, believes that one does not attempt to average several opinions. One simply identifies an appropriate expert, solicits his opinion, and goes on with the analysis. For those who mistrust the opinion of a single expert, however, techniques for achieving concensus among experts may be attractive (e.g. Zuboy 1981).

Applying the Decision Rule

The final step in MAUT analysis (Table 1) is to calculate the aggregate utility value for each yield option (eq. 2). Each weight by score product for an attribute is a utility value for that option on that attribute. The performance of any option may be examined over any subset of attributes as well as over the whole set. I have, therefore, aggregated utilities over biological, economic, and social attributes for each fishery as well as over the whole set of attributes (Table 8).

The outcome of the analysis for the Skeena salmon fishery is quite straightforward. All the yield alternatives involving a reduction in effort scored higher than the policies involving the current level of effort. For all alternatives except status quo allocation at current levels of effort the scores were well distributed among the biological, economic, and social attributes. Somewhat surprisingly, however, the alternative involving elimination of the lure fishery scored much better on virtually all attributes than the other alternatives. This result is, of course, a consequence of the set of weights and scores that I used in the analysis. I doubt that the lure fishermen would agree with the wisdom of any analysis that predicted an increase in general welfare associated with their elimination. This result may be spurious, since I have not attempted to determine rigorously the preference structure of stakeholders in this fishery. The result is also dependent on my assumption that greater control could be exercised over coho, chinook, and steelhead escapements in the absence of the lure fishery. It is important to remember, however, that I did not conduct the analysis with this outcome in mind. It is also important to ask how much of a change in attribute weights would be required to make the "no lure fishery" option less attractive than the "status quo" option. If the division of preference among the biological (0.45), economic (0.30), and social (0.25) dimensions is kept the same, then putting all of the economic and social weight into attributes associated with the lure fishery still does not make the "no lure fishery" option less attractive than the status quo. Thus, a pretty fundamental change in preference structure would be required in order to change the outcome of the analysis with respect to the desirability of the lure fishery.

TABLE 8. Aggregate utility scores for six yield alternatives in the Skeena salmon fishery (Table 6) and the nine yield alternatives in the New England herring fishery (Table 7). Scores are aggregated across biological, economic, and social attributes as well as across all attributes.

Policies	Utility scores aggregated across: Biological attributes	Economic attributes	Social attributes	All attributes
	Skeena salmon fishery			
Alternative				
1	0.0	6.0	1.8	7.8
2	5.8	4.6	5.8	16.2
3	19.0	11.6	12.6	43.2
4	27.3	21.0	17.5	65.8
5	35.0	22.1	22.0	79.1
6	43.4	22.0	22.0	87.4
	New England herring fishery			
Alternative				
1	41.0	17.7	8.1	66.8
2	26.6	14.7	10.8	52.1
3	4.1	8.4	6.7	19.2
4	37.7	20.3	11.3	69.3
5	29.2	17.3	12.8	59.3
6	2.0	9.9	9.6	21.5
7	34.8	21.7	13.2	69.7
8	29.2	18.9	15.8	62.9
9	0.0	10.3	11.7	22.0

The results for the herring fishery are less straightforward. All the MEY options scored best. MEY in the juvenile only fishery had the highest score, but only marginally higher than MEY in the mixed juvenile/adult fishery. All MSY options also scored well, and again MSY in the juvenile only fishery scored highest among these options. The bionomic equilibrium options all scored poorly relative to the other options. What the analysis suggests is that there is little to choose among the MEY and MSY options for any of the allocation policies examined and that some other attributes or some other decision rule would have to be invoked to distinguish among these options. It is a little surprising that the "adult only" option scored relatively poorly considering the potential improvement in yield associated with this option. The apparent social and economic advantages of the juvenile or mixed juvenile/adult fishery, however, outweighed the loss of production in these alternatives.

Discussion

A detailed evaluation of the yield options for either the Skeena salmon fishery or the New England herring fishery is probably not warranted. The attribute weights and the scores used in the analysis are preliminary and are intended to illustrate the method rather than solve the OY problem in these fisheries. The preference weights and the scores are, nevertheless, plausible so the analysis results should not merely be dismissed as unrealistic. The analysis suggests that a reduction in the lure fishery on the Skeena River salmon stocks would significantly benefit the hard-pressed runs of chinook, coho, and steelhead. This result is consistent with current thinking among regional managers in DFO. But the analysis also suggests that cutting back the lure fishery would increase the general welfare of the region, although, of course, the lure fishermen would suffer. In a similar way the analysis of the New England herring fishery suggests that a reduction in fishing effort would incrase the general welfare of the region, although some fishermen would presumably suffer.

The degree to which a few should suffer so that others might benefit has always been a contentious question in fisheries management. In part, this relates to the trade-off between short- and long-term benefits mentioned earlier. One decision analytic approach to this trade-off is through appropriate choice of decision rule. The rule that I chose in the analysis of OY for the salmon and herring fisheries was to maximize aggregate utility. This rule is indifferent to the negative side effects of a policy because it reacts only to the net positive effects. Other rules are possible, in particular, rules that seek to minimize the sum of negative effects of a policy. Suppose, for example, that I had used the following scoring procedure:

$$(3) \quad S_{ij} = I_j - A_{ij}$$

where I_j is the ideal score for the jth attribute and A_{ij} is the actual score for the ith solution on the jth attribute. Equation 1 then becomes an analytic solution that is sensitive to departures from an idealized set of attribute scores. The yield solution that minimizes U when $p = \infty$ is a solution that minimizes the negative effects of a set of attributes (Duckstein and Opricovic 1980). In practice it is desirable to use several decision rules in evaluating any set of management alternatives so that the positive and negative effects of each can be explored.

The MAUT techniques that I have described are among the less sophisticated of the decision analytical methods. Considerably more sophisticated, and intellectually more rigorous, techniques exist. The sophisticated methods have the disadvantage, however, that they are more difficult for the decision maker to identify with. I consider that there are four principle advantages to applying simple MAUT techniques to the solution of complex fishery management problems like OY. First, the techniques are specifically designed to help with the practical solution of multiattribute decision problems. This is the class of problems to which OY and related fisheries management problems belong. Second, the techniques are designed to mimic the natural decision making process so that they are intellectually appealing and understandable to the decision maker. Third, they provide a structured analytic framework for quantitatively evaluating alternative solutions. This means that any decision supported by such an analysis is not only transparent but is also amenable to sensitivity analysis. Fourth, the techniques permit a broad range of types of information and levels of measurement to be brought together into a single objective decision rule. Differences in measurement units, scales, accuracy, and precision are not a problem, so far as the analysis is concerned. Tangible and intangible attributes can be incorporated with equal ease. Thus, the manager can bring all the information at his disposal, or any set of it, to bear on the problem and can feel confident that decisions will not be flawed because he was unable to incorporate objectively some piece of information.

Value judgements are clearly a large part of the application of MAUT. In the past, fishery managers have shunned any appearance of making value judgements. Of course, they were making value judgements all the time. The decision to manage to MSY is a value judgement. Every regulation to control fishing effort has consequences for allocation of which managers were well aware, and so their application involved a value judgement. It was, however, considered dangerous to be honest about such side effects of resource management. The MAUT techniques not only provide a way to bring the value judgements inherent in any decision about OY out in the open, but also provide a way to absolve the manager of the criticism that he is imposing his values on the industry. Provided the manager is diligent about identifying the important stakeholder groups and capturing their attribute weights then he can reasonably argue that everyone's views were taken into account in determining OY.

There is no escape from the fact that determining OY requires value judgements. The analytic models that I have described, however, provide an objective framework for assessing the value judgements that determine an optimum. Like other analytic models, MAUT models can be abused. For example, it is possible to backtrack from a predetermined solution to a set of attributes and weights that will support that solution. The transparent nature of decisions based on MAUT techniques, however, and the fact that they can be subjected to sensitivity analysis makes such abuse difficult and potentially unproductive. These techniques capture the spirit of the OY concept within a rigorous analytic framework yet they also provide the flexibility that the decision maker needs to deal with the multifaced nature of fisheries management.

Acknowledgments

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BOOK REVIEWS/COMPTES RENDUS

Aquatic Toxicology. *Edited by J. O. Nriagu. John Wiley & Sons, New York, NY. 1983. 525 p. $90 (U.S.).*

One cannot tell what kind of book this is from its very broad title. It is a collection of 19 chapters by different authors covering discrete topics; it is not a textbook covering all the principles and approaches in aquatic toxicology.

Nine of the chapters are straight reviews of the literature and I welcome them wholeheartedly. Every time I see the results of a computer search of the literature, with its hundreds of relevant papers, I feel that each aquatic toxicologist should stop research and write reviews of what has already been done and what it all means. Some of the authors in this book have done excellent integrative reviews of that kind. For example, Borgmann tackles the complexity of metal speciation in water, applies a little theory and empiricism, and emerges with predictive straight-line relationships between toxicity of the metals and pH of the water. He even manages to fit into single regressions the results for various species of fish provided by diverse authors. He ends with a plausible hypothesis for the modifying effect of pH. Other reviews that are heavy on interpretation are Heckman on ecological implications of pest-control strategies and Hara et al. on behavioural responses to pollutants and the underlying physiological mechanisms. I will break the rule about not listing contents of a book, because the rapidity with which you rush out to find it will depend on how closely the chapters overlap your interests. The other reviews cover vanadium, crude oil, the insecticide Matacil, physiological categories of effect with emphasis on bioenergetics, adaptation to pollutants, and membrane functioning with particular reference to transport of toxicants. A few of these chapters needed general interpretive summaries.

Another broad category of chapters described particular methods. Four of these were about algae and varied from general coverage of responses to detailed descriptions of a laboratory procedure for one species. There was a useful comparison of results from static and continuous-culture techniques. Four more chapters dealt specifically with the use of flatworms, ciliates, cytogenetics, and ATPase or adenylate energy charge. Whole-organism responses among these tiny species have real advantages in speed, cost, and meaningfulness, and this was clearly brought out. However, as mentioned at the beginning, the "pot-luck" coverage by the chapters meant that some useful organisms were not mentioned (e.g. *Daphnia*), nor was there any coverage of recent rapid procedures with early life-stages of fish.

Other chapters were essentially major presentations of work from individual laboratories: structure–activity predictions for nitrogen heterocyclic molecules; metal speciation and toxicity to microbiota; and mercury dynamics in microcosms.

Any scientist concerned with effects of water pollutants will find something of interest in this collection. I found three chapters of direct and immediate value, and almost all chapters had useful parts. Certainly the book should be in the library of any laboratory working on aquatic environmental problems, although an individual would no doubt hesitate to buy it for the price listed above. The book is handsomely done and I did not notice any errors in printing or technical presentation.

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The Chartmakers — A History of Nautical Surveying in Canada. *Stanley Fillmore and R. W. Sandilands. Toronto. NC Press. 1983. viii, 255 p., bibliography, index. 95 colour photos, charts, black and white illustrations and photographs. ISBN 0919601-92-8. $34.95.*

The Chartmakers is a timely work in the light of today's offshore rights and land claims in Canada. Early explorers such as Champlain, Cook, and Vancouver produced charts that helped establish sovereignty for both England and France in Canada.

On the west coast of Canada, systematic charting began with the British Admiralty survey vessels in the mid-nineteenth century. This was in response to several important developments. The 1825 Anglo–Russian treaty saw the Russians claim right to the land north of latitude 51°N for a distance of 100 miles from shore. This soon became of concern to both British and Americans because of their developing settlement pattern towards the west. There was also the continuing need to find the elusive Northwest Passage, which attracted no fewer than 33 British survey and exploration vessels to the Arctic area between 1849 and 1859.

Today, oil exploration such as in the Beaufort Sea area, Inuit activities and claims, as well as defence considerations, have lent greater importance to charting the Arctic region. On the east coast, oil explorations and fishing rights constantly require updating charts. The needs of industry and commerce, of which shipping is an essential part, have resulted in the production of up-to-date charts in the Great Lakes area.

It is with this in mind that the authors combined their respective talents, Fillmore the professional writer, and Sandilands the hydrographer, to produce a fascinating description of an important Canadian Government service which is of benefit to all. Although readers might be acquainted with a number of good articles on Canadian charting, they will be hard put to find a book such as this.

The Chartmakers traces the enormous task of charting along the shores of three oceans with more coastline and navigable rivers than any other country. Some early historical background leads up to the appointment in 1883 of Captain John George Boulton as Canada's first chief hydrographer and the subsequent

establishment of the Canadian Hydrographic Service under the direction of William J. Stewart.

Technical terms such as Portolan maps, rhumb lines, astrolabes, the traverse board, and mercator projections are carefully and simply explained, as are latitude, longitude, and the importance of the chronometer. The human element is not neglected, since the men of the service and their tasks are noted. The origin of the term "limey" for British sailors is explained as is the former dreaded seafaring disease of scurvy.

The impact of a number of important devices on the progress of charting has not been overlooked. It was in 1928 that Canadian hydrographers first used the gyroscopic compass, which keeps its compass card constantly aligned with magnetic north. The echo sounder followed soon after. Then, in 1961, a sounder capable of working through ice became a boon to northern charting. In the logical progression of the book, chart compilation, together with the uses and examples of symbols, reveals the work in the "office" in contrast with the "fieldwork." Another important part of charting is the selection and submission of suitable names. These must be carefully screened so that native spellings are correct, duplication is avoided, and nobody offended. When it is realized that there are now more than 1000 navigational charts and about 500 special charts of the Canadian Hydrographic Service, the magnitude of the work can be appreciated.

The serious reader at this point might wish to glance at an index of the charts produced. It would have been helpful if a reduced scale version of the standard index could have been produced in the book. As it is, one must refer to separate sheets obtainable from the Canadian Hydrographic Service or from authorized chart dealers.

A section devoted to the ships of the hydrographic service reveals the problems and successes over the years. The first vessel purchased was a 20-yr-old American tug. Important survey vessels such as the *Acadia*, the *Wm. J. Stewart*, and the *Baffin*, built especially for the hydrographers, are among the best known. Wartime activities saw the *Wm. J. Stewart* perform work on either coast. This was the time when the "egg route" charts were made, of which little was generally known.

Although the ships are illustrated, some readers might feel that there is an overpowering number of single-page, colour pictures without individual captions, and are therefore more colourful than informative. However, they do create a certain mood.

Of sustaining interest are the various facts given. For example, the chart for Prince Rupert was the first all-Canadian one of the west coast based on surveys completed in 1909. The first ship casualty of the Service was the *Crissie C. Thomey* in June 1913, and the first ship constructed for hydrographic work was CGS *Lillooet* in 1908. There are also numerous anecdotes that are amusing and, without so many words, it soon becomes clear that the role of the hydrographer is to provide information for mariners on underwater hazards to navigation as well as on tides and currents, which is published in charts, *Sailing Directions*, and *Tide and Current Tables*.

Since only about half of Canada's waterways are surveyed adequately for present requirements and only a portion of the Arctic waters have been surveyed, there is still much work for the Canadian Hydrographic Service to accomplish as time and funding permit.

All in all, Stanley Fillmore and R. W. "Sandy" Sandilands have made the most of producing fascinating reading for anyone interested in ships, the sea, exploration, challenge, the environment, and Canada's history and geography. There is evident a nice balance among all the aspects of Canadian hydrographic surveying discussed and the reader will be left with the comforting knowledge that much is being done to ensure the safety of vessels, both large and small, in our Canadian waters.

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Articles

Genetic variation in populations of steelhead trout (*Salmo gairdneri*) in British Columbia **E. A. Parkinson**

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Inorganic nitrogen uptake in oligotrophic Lake Taupo, New Zealand **J. C. Priscu and L. R. Priscu**

Genetic effect on the dynamics of a model of pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon **W. W. Smoker**

Spawning characteristics of sympatric populations of steelhead trout (*Salmo gairdneri*): evidence for partial reproductive isolation **S. A. Leider, M. W. Chilcote, and J. L. Loch**

Relationships between the partitioning of trace metals in sediments and their accumulation in the tissues of the freshwater mollusc *Elliptio complanata* in a mining area **A. Tessier, P. G. C. Campbell, J. C. Auclair, and M. Bisson**

Particulate allochthonous inputs: relationships with stream size in an undisturbed watershed **M. E. Conners and R. J. Naiman**

Efficiency estimates for various quadrat sizes used in benthic sampling **J. D. Pringle**

Socioeconomic aspects of sea cage salmon farming in the Maritimes **N. B. Ridler**

Changes in the benthic community of a small precambrian lake following the introduction of yellow perch, *Perca flavescens* **J. R. Post and D. Cucin**

Tri- and tetra-chloroveratrole, metabolites produced by bacterial *O*-methylation of tri- and tetra-chloroguaiacol: an assessment of their bioconcentration potential and their effects on fish production **A. H. Neilson, A.-S. Allard, S. Reiland, M. Remberger, A. Tärnholm, T. Viktor, and L. Landner**

Intakes as sampling locations for investigating long-term trends in zooplankton populations **M. S. Evans and L. E. Flath**

Comparison of *N*,*N*-dimethylformamide, dimethyl sulfoxide, and acetone for extraction of phytoplankton chlorophyll **B. J. Speziale, S. P. Schreiner, P. A. Giammetteo, and J. E. Schindler**

Notes

Effect of an intertidal mudflat on plankton of the overlying water column **D. J. Carlson, D. W. Townsend, A. L. Hilyard, and J. F. Eaton**

Stress-related changes in lake whitefish (*Coregonus clupeaformis*) associated with a hydroelectric control structure **M. A. Barnes, G. Power, and R. G. H. Downer**

Perspectives

Need for development of epidemiology for chemically induced diseases in fish in Canada **M. Gilbertson**

Book reviews/Comptes rendus

Lakes of the warm belt (Colette Serruya and Utsa Pollingher) **R. G. Wetzel**

Announcements/Avis

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Volume 41, No. 9, September 1984

Volume 41, nº 9, septembre 1984

Articles

Determination of corticosteroids in fish plasma by high performance liquid chromatography: evaluation of the method using striped bass (*Morone saxatilis*) R. B. MacFarlane **1280–1286**

Variable gut residence time: problems in inferring feeding rate from stomach fullness of a mysid crustacean P. A. Murtaugh **1287–1293**

Selective exploitation of capelin (*Mallotus villosus*) eggs by winter flounder (*Pseudopleuronectes americanus*): capelin egg mortality rates, and contribution of egg energy to the annual growth of flounder K. T. Frank and W. C. Leggett **1294–1302**

Zooplankton and potential fish production in Lake Ontario U. Borgmann, H. Shear, and J. Moore **1303–1309**

Influence of temperature and light on rates of inorganic nitrogen transport by algae in an arctic lake S. C. Whalen and V. Alexander **1310–1318**

Pacific salmon (*Oncorhynchus*) migrations: orientation versus random movement T. P. Quinn and C. Groot **1319–1324**

Generalized Stock Reduction Analysis D. K. Kimura, J. W. Balsiger, and D. H. Ito **1325–1333**

Lethal and sublethal toxicity of drilling fluids to larvae of the American lobster, *Homarus americanus* J. G. S. Derby and J. M. Capuzzo **1334–1340**

Selection of external tags for marking Atlantic herring (*Clupea harengus harengus*) B. S. Nakashima and G. H. Winters **1341–1348**

Demographic consequences of precocious maturation of Atlantic salmon (*Salmo salar*) R. A. Myers **1349–1353**

Extracellular protease, extracellular haemolysin, and virulence in *Aeromonas salmonicida* J. L. Hackett, W. H. Lynch, W. D. Paterson, and D. H. Coombs **1354–1360**

Statistical inference in life-table experiments: the finite rate of increase P. J. Rago and R. M. Dorazio **1361–1374**

Influence of chlorine on the susceptibility of striped bass (*Morone saxatilis*) to *Vibrio anguillarum* F. M. Hetrick, L. W. Hall, Jr., S. Wolski, W. C. Graves, B. S. Roberson, and D. T. Burton **1375–1380**

Notes

Comparisons of growth in weight of temperate and tropical marine fish counterparts R. R. C. Edwards **1381–1384**

Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*) J. L. Atkinson, J. W. Hilton, and S. J. Slinger **1384–1386**

Growth and gonadal development in triploid landlocked Atlantic salmon (*Salmo salar*) T. J. Benfey and A. M. Sutterlin **1387–1392**

Perspectives

Multiattribute analysis and the concept of optimum yield M. C. Healey **1393–1406**

Book reviews/Comptes rendus

Aquatic toxicology (edited by J. O. Nriagu) J. B. Sprague **1407**

The chartmakers — A history of nautical surveying in Canada G. Castle **1407–1408**

Coming next month/À paraître le mois prochain **1409**

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